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LIST OF PROTEINS

4-1BBL	Caspase-3	sFlt-1 (D3)	IL-2	MEC	sRANK
4-1BB Receptor	Caspase-6	sFlt-1 (D4)	IL-3	Mek-1	sRANKL
6 Ckine	CD4	sFlt-1 (D5)	IL-4	MIA	RANTES
ACAD8	CD14	sFlt-1 (D7)	sIL-4 Receptor	Midkine	RELM- α
ACAT2	CD22	Flt3-Ligand	IL-5	MIG / CXCL9	RELM- β
gAcrp30/Adipolean	CD40 Ligand / TRAP	sFlt-4	IL-6	MIP-1 α / CCL3	Resistin
Activin A	CD95 / sFas Ligand	sFlt-4/ Fc Chimera	sIL-6 Receptor	MIP-1 β / CCL4	RPTP β
ACY1	CD105 / Endoglin	Follistatin	IL-7	MIP-3 / CCL23	RPTP γ
ADAT1	CHIPS	FSH	IL-8 (72 a.a.)	MIP-3 α / CCL20	RPTP μ
Adiponectin	CNTF	Fractalkine/ CX3C	IL-8 (77 a.a.)	MIP-3 β / CCL19	SCF
ADRP	Collagen	G-CSF	IL-9	MIP-4 (PARC) / CCL18	SCGF- α
AITRL	CREB	α -Galactosidase A	IL-10	MIP-5 / CCL15	SCGF- β
Akt1	CTACK/CCL27	Galectin-1	IL-11	MMP-3	SDF-1 α
Alpha-Feto Protein (AFP)	CTGF	Galectin-3	IL-12	MMP-7	SDF-1 β
Alpha-Galactosidase A	CTGFL/WISP-2	Gastrointestinal CA	IL-13	MMP-13	Secretin
Angiopoietin-1 (Ang-1)	CTLA-4/Fc	GCP-2	IL-13 analog	Myostatin	SF20
Angiopoietin-2 (Ang-2)	CXCL16	GDF-3	IL-15	Nanog	SHP-2
Angiostatin K1-3	Cytokeratin 8	GDF-9	IL-16 (121 a.a.)	NAP-2	STAT1
Annexin-V	DEP-1	GDF-11	IL-16 (130 a.a.)	Neurturin	c-Src
apo-SAA	Desmopressin	GDNF	IL-17	NFAT-1	TACI
Apolipoprotein A-1	Disulfide Oxidoreductase	GLP-1	IL-17B	beta-NGF	TARC
Apolipoprotein E2	E-selectin	Glucagon	IL-17D	NOGIN	TC-PTP
Apolipoprotein E3	ECGF	Goserelin	IL-17E	NOV	TECK
Apolipoprotein E4	EGF	GM-CSF	IL-17F	NP-1	TFF2
APRIL	Elafin/SKALP	GPBB	IL-19	NT-1/BCSF-3	TGF- α
Artemin	EMAP-II	GRO α	IL-20	NT-3	TGF- β 1
ATF2	ENA-78	GRO β	IL-22	NT-4	TGF- β 2
Aurora A	Endostatin	GRO γ	IL-31	Ocreotide	TGF- β 3
Aurora B	Enteropeptidase	GRO/MGSA	Insulin	Oncostatin M	Thymosin α 1
BAFF	Eotaxin	Growth Hormone	IP-10	Osteoprotegerin (OPG)	sTIE-1/Fc Chimera
BAFF Receptor	Eotaxin-2	Growth Hormone BP	JE	OTOR	sTIE-2/Fc Chimera
BCA-1 / BLC / CXCL13	Eotaxin-3 (TSC)	GST-p21/WAF-1	JNK2a1	Oxytocin	TL-1A
BCMA	EPHB2	HB-EGF	JNK2a2	p38- α	TNF- α
BD-1	EPHB4	HCC-1	KC / CXCL1	Parathyroid Hormone	TNF- β
BD-2	Eptifibatide	HGF	KGF	PDGF-AA	sTNFR1
BD-3	Erk-2	Histidyl-tRNA synthetase	L-asparaginase	PDGF-AB	sTNFR2
BDNF	Erythropoietin (EPO)	Histrelin	LAG-1	PDGF-BB	TPO
Bivalirudin	Exodus-2	HRG1- β 1	LALF Peptide	Persephin	TRAIL/Apo2L
BMP-2	Fas Ligand	I-309	LAR-PTP	PF-4	sTRAIL R-1 (DR4)
BMP-4	Fas Receptor	I-TAC	LC-1	PIGF-1	sTRAIL R-2 (DR5)
BMP-7	FGF-1 (acidic)	IFN- α	LBP	PIGF-2	TSH
BMP-13	FGF-2 (basic)	IFN- α A	LD-78 β	PKA α -subunit	TSLP
sBMPR-1A	FGF-4	IFN- α 2a	LDH	PKC- α	TWEAK
Brain Natriuretic Protein	FGF-5	IFN- α 2b	LEC/NCC-4	PKC- γ	TWEAK Receptor
BRAK	FGF-6	IFN- β	Leptin	Pleiotrophin	Urokinase
Breast Tumor Antigen	FGF-7/ KGF	IFN- γ	LIGHT	PLGF-1	VEGF121
C5a	FGF-8	IFN-Omega	LIX	Polymyxin B (PMB)	VEGF145
C5L2 Peptide	FGF-9	IGF-I	LKM	PRAS40	VEGF165
C-10	FGF-10	IGF-II	LL-37	PRL-1	VEGF-C
C-Reactive Protein	FGF-16	proIGF-II	Lymphotactin	PRL-2	VEGF-C 1525
C-Src	FGF-17	IGFBP-1	sLYVE-1	PRL-3	EG-VEGF
Calbindin D-9K	FGF-18	IGFBP-2	M-CSF	Prokineticin-2	VEGF-E
Calbindin D-28K	FGF-19	IGFBP-3	MCP-1 (MCAF)	Prolactin	HB-VEGF-E
Calbindin D-29K	FGF-20	IGFBP-4	MCP-2	Protirelin	sVEGFR-1
Calmodulin	sFGFR-1 (IIIc) / Fc Chimera	IGFBP-4	MCP-3	PTHrP	sVEGFR-2
Calcitonin Acetate	sFGFR-2 (IIIc) / Fc Chimera	IGFBP-5	MCP-4	PTP1B	sVEGFR-3
Carbonic Anhydrase III	sFGFR-3 / Fc Chimera	IGFBP-6	MCP-5	PTP-IA2	WISP-1
Carcino-embryonic Antigen	sFGFR-4 / Fc Chimera	IGFBP-7	MDC (67 a.a.)	PTP-MEG2	WISP-2
Cardiotrophin-1	sFlt-1 (native)	IL-1 α	MDC (69 a.a.)	PTP-PEST	WISP-3
		IL-1 β	MDH		WNT-1

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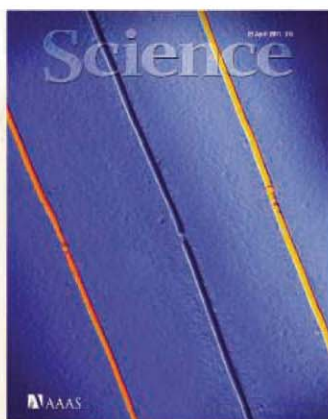
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COVER

False-colored atomic force microscopy image of three carbon nanotubes on a silicon dioxide substrate (composite of scans on three devices). The middle nanotube is disconnected by a ~40-nanometer gap; the outer nanotubes have similar nanogaps bridged by a phase-change material (PCM) bit. Such small bits can be switched by voltage pulses with energy consumption that is one hundred times lower than that of modern PCM data storage. See page 568.

Image: Alex Jerez, Feng Xiong, and Eric Pop, University of Illinois Urbana-Champaign

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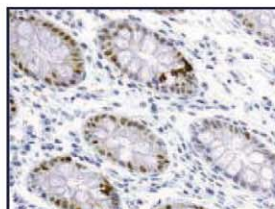
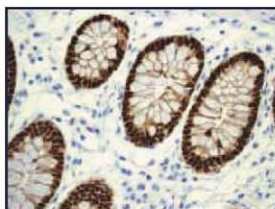
Nanomolar ($\sim 10^{-9}$ kD M)

APPLICATIONS

Westerns, ELISA, Flow Cytometry, IP, IHC, ICC - Excellent results in IHC

Westerns, ELISA, Flow Cytometry, IP - Not suitable for mouse studies

IHC



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REVIEW

- 555 Microresonator-Based Optical Frequency Combs
T. J. Kippenberg et al.

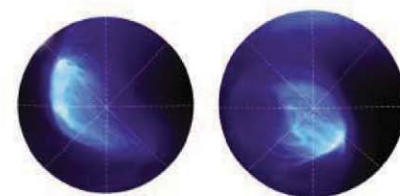
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- 564 Orbital-Independent Superconducting Gaps in Iron Pnictides
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- 568 Low-Power Switching of Phase-Change Materials with Carbon Nanotube Electrodes
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- 580 Surface-Generated Mesoscale Eddies Transport Deep-Sea Products from Hydrothermal Vents
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Deep-reaching eddies transport heat and material hundreds of kilometers from the northern East Pacific Rise.

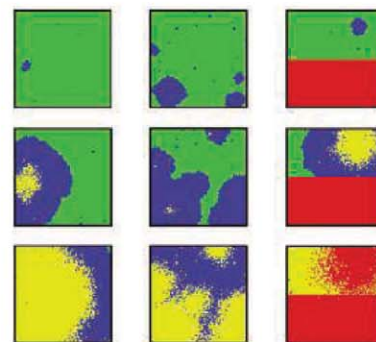
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- 600 Trans-Endocytosis of CD80 and CD86: A Molecular Basis for the Cell-Extrinsic Function of CTLA-4
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An inhibitory T cell receptor acts by stripping activating ligands off dendritic cells.
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Methylation of the bacterial ribosome by two methyltransferases proceeds by an unusual radical mechanism.
>> *Perspective p. 544; Science Express Report by A. K. Boal et al.*
- 608 A Crystal Structure of the Complex Between Human Complement Receptor 2 and Its Ligand C3d
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The topology of this molecular interface provides a foundation for the design of therapeutics against autoreactive B cells.



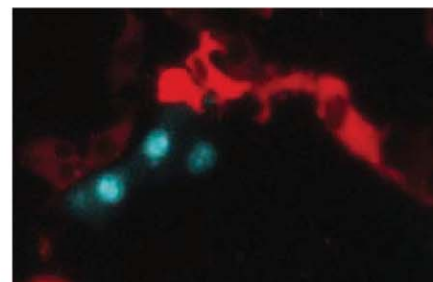
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Structural Basis for Methyl Transfer by a Radical SAM Enzyme

A. K. Bøal et al.

An enzyme harnesses methyl donation and reductive cleavage of its cofactor, within a single active site, to methylate RNA.

10.1126/science.1205358

>> *Perspective p. 544; Report p. 604*

Human Cytomegalovirus Directly Induces the Antiviral Protein Viperin to Enhance Infectivity

J.-Y. Seo et al.

Human cytomegalovirus uses a host-encoded antiviral protein to its own advantage.

10.1126/science.1202007

Early Warnings of Regime Shifts: A Whole-Ecosystem Experiment

S. R. Carpenter et al.

High-frequency monitoring of manipulated and reference lakes enabled early detection of subsequent catastrophic regime shift.

10.1126/science.1203672

Spin-Liquid Ground State of the $S = 1/2$ Kagome Heisenberg Antiferromagnet

S. Yan et al.

Numerical calculations reveal that the true ground state of a frustrated two-dimensional system is a gapped spin liquid.

10.1126/science.1201080

Imaging Doped Holes in a Cuprate Superconductor with High-Resolution Compton Scattering

Y. Sakurai et al.

Inelastic x-ray scattering probed the doping dependence of the electronic environment within a cuprate superconductor.

10.1126/science.1199391

The 2010 M_w 8.8 Maule Mega-Thrust Earthquake of Central Chile, Monitored by GPS

C. Vigny et al.

Rupture kinematics of this very large earthquake were obtained from high-resolution global positioning system data.

10.1126/science.1204132

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Replication of "The Perception of Rational, Goal-Directed Action in Nonhuman Primates" by J. N. Wood and M. D. Hauser.

10.1126/science.1202596

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Comment on "Erosion of Lizard Diversity by Climate Change and Altered Thermal Niches"

S. Clusella-Trullas and S. L. Chown

Full text at www.sciencemag.org/cgi/content/full/332/6029/537-a

Response to Comment on "Erosion of Lizard Diversity by Climate Change and Altered Thermal Niches"

B. Sinervo et al.

Full text at www.sciencemag.org/cgi/content/full/332/6029/537-b

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Highlights From Our Daily News Coverage

Expert Panel: Central U.S. Faces

Real Threat of Quake

Millions in the American heartland face a genuine, if uncertain, seismic risk.

<http://scim.ag/new-madrid>

2010's Volcano-Induced Air Travel Shutdown Was Justified

Study finds that Eyjafjallajökull ash could have harmed aircraft.

<http://scim.ag/air-shutdown>

Fire Ants Surf Floods on Rafts of Their Own Bodies

Thousands of ants link together to trap air and repel water.

<http://scim.ag/ant-rafts>

SCIENCE SIGNALING

www.sciencesignaling.org

The Signal Transduction Knowledge Environment

26 April issue: <http://scim.ag/ss042611>

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W. Wong

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RESEARCH ARTICLE: Thioredoxin Mediates Oxidation-Dependent Phosphorylation of CRMP2 and Growth Cone Collapse

A. Morinaka et al.

Repulsive guidance signaling triggers the collapse of neuronal growth cones through a mechanism involving oxidation and phosphorylation.

PERSPECTIVE: PI3K and Chemotaxis—A Priming Issue?

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PERSPECTIVE: Sorting the Signals from the Signals in the Noisy Environment of Inflammation

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JOURNAL CLUB: Analyzing the Anatomy of Integrin Adhesions

A. Byron

The spatial organization of integrin adhesions provides insight into their molecular functions.

SCIENCE CAREERS

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A Twin Career Story

E. Pain

Identical twin brothers Alan and Steven Davy are pursuing similar research interests while also seeking to differentiate themselves.

<http://scim.ag/twinstory>

Q&A: Taking Mathematics to Heart

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Mathematician John Wesley Cain works with clinicians, physicists, and engineers in a field called cardiac electrophysiology.

<http://scim.ag/math2heart>

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Integrating Medicine and Science

27 April issue: <http://scim.ag/stm042711>

RESEARCH ARTICLE: Establishment of Antitumor Memory in Humans Using in Vitro-Educated CD8⁺ T Cells

M. O. Butler et al.

Antitumor CD8⁺ T cells educated in vitro can induce antitumor responses in humans.

REPORT: Differential Metabolic Impact of Gastric Bypass Surgery Versus Dietary Intervention in Obese Diabetic Subjects Despite Identical Weight Loss

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PERSPECTIVE: Two Roads Diverge—Weight Loss Interventions and Circulating Amino Acids

R. E. Gerszten and T. J. Wang

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PERSPECTIVE: The NCGC Pharmaceutical Collection—A Comprehensive Resource of Clinically Approved Drugs Enabling Repurposing and Chemical Genomics

R. Huang et al.

The NIH Chemical Genomics Center houses a database and a library of approved drugs.

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Science Policy News and Analysis

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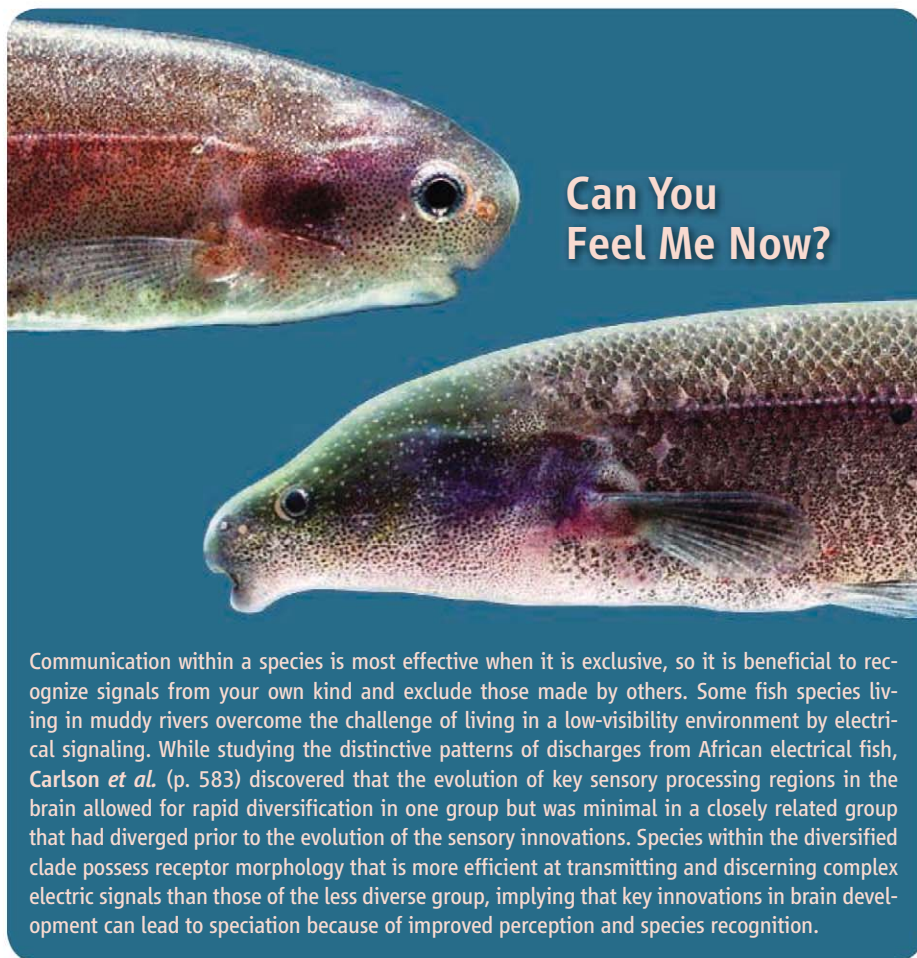
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Communication within a species is most effective when it is exclusive, so it is beneficial to recognize signals from your own kind and exclude those made by others. Some fish species living in muddy rivers overcome the challenge of living in a low-visibility environment by electrical signaling. While studying the distinctive patterns of discharges from African electrical fish, **Carlson *et al.*** (p. 583) discovered that the evolution of key sensory processing regions in the brain allowed for rapid diversification in one group but was minimal in a closely related group that had diverged prior to the evolution of the sensory innovations. Species within the diversified clade possess receptor morphology that is more efficient at transmitting and discerning complex electric signals than those of the less diverse group, implying that key innovations in brain development can lead to speciation because of improved perception and species recognition.

Putting the F in Friedel-Crafts

The Friedel-Crafts class of reactions, among the oldest and most broadly applied in organic chemistry, form carbon-carbon bonds between aromatic rings and a variety of non-aromatic substituents, such as alkyl groups. Generally, a metal complex is used to activate chlorinated or brominated precursors of these substituents, but by using silicon-based reagents to activate a fluorinated precursor, **Allemann *et al.*** (p. 574) extend the reaction to coupling of two different aromatic sites, leading to efficient formation of elaborate polycyclic structures. The method relies on the unusual strength of silicon-fluorine bonds as a driving force.

Insulator to Order

Unlike simple band-gap insulators, bulk topological insulators permit movement of charges on their boundaries while acting as insulators in their interior. Both types of insulators usually occur in different families of compounds. Now, **Xu *et al.*** (p. 560, published online 31 March;

see the Perspective by **Fiete**) can promote transition between a direct band gap and topological insulator within a bulk bismuth-containing solid by changing the relative ratio of its sulfur and selenium content. This results in the tuning of the spin-orbit interaction and a topological phase transition in which the material $\text{TlBi}(\text{S}_{1-x}\text{Se}_x)_2$ exhibits a 180-degree inversion of its Fermi surface spin pattern as the Fermi energy is tuned through the zero-electron density point. This spin texture inversion may lead to devices with novel quantum properties.

Changing as Little as Possible

Phase-change materials can be used in memory devices because they change a physical property, such as resistivity or optical reflectivity, depending on whether they are in an amorphous or crystalline state. These changes are thermally driven, but, for memories based on resistivity changes, one hurdle has been to reduce the amount of power needed to melt these materials. **Xiong *et al.*** (p. 568, published

online 10 March; see the Perspective by **Salinga and Wuttig**) created gaps in single-walled and small-diameter multiwalled carbon nanotubes and then coated these electrodes with a thin film of the phase-change material $\text{Ge}_2\text{Sb}_2\text{Te}_5$. Phase changes occurred only for the material embedded in the gaps, which, when compared with current device configurations, helps reduce the power required by two orders of magnitude.

Improving Organic LEDs

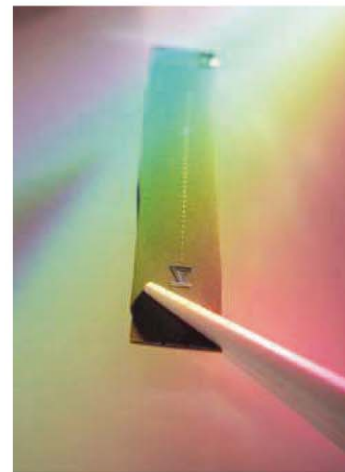
Organic light-emitting diodes (OLEDs) could form the basis of faster, lower-powered displays, by comparison with liquid crystal displays that require a lighting source and have inherent viewing-angle limitations. However, OLEDs require higher driving voltages, and this has limited the number of materials that can be used for the active matrix backplane. **McCarthy *et al.*** (p. 570) show that single-walled carbon nanotubes can be used to fabricate vertical field-effect transistors to integrate with OLEDs and generate efficient light emission across the color spectrum.

Frequency Combs on a Chip

Precisely spaced spectral lines that span a broad range of wavelengths to form a frequency comb have been used in a variety of applications, including precision metrology and spectroscopy, molecular fingerprinting, and astronomical observations. These combs generally have been produced using pulsed laser systems, which require expensive equipment.

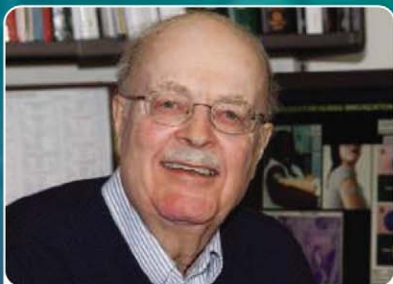
Kippenberg *et al.* (p. 555) review recent developments

using microresonators to generate frequency combs. This alternative technology means that not only can the comb source be reduced to chip-size scale, the frequency ranges can be controlled and tuned by the size and shape of the resonators, thereby opening up the range of possible applications.



Continued on page 512

“A dream told me to do it.”



Carl R. Alving, M.D.
Chief of the Department of Adjuvant & Antigen Research, Division of Retrovirology at the Walter Reed Army Institute of Research
AAAS member

*Dr. Carl Alving
on his inspiration
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the vaccine patch.*

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This Week in *Science*

Continued from page 511

Changing Vortex

Like Earth’s atmosphere, the atmosphere of Venus features large-scale cyclonic circulation patterns, or vortices, at its poles. Based on long-term, high-resolution data from the European Space Agency Venus Express mission, **Luz *et al.*** (p. 577, published online 7 April) show that the southern polar atmospheric circulation is more complex than was previously thought. The center of the south polar vortex is slightly displaced from the solid planet’s pole of rotation, and it moves around the pole with a period of 5 to 10 Earth days. These findings should contribute to improved models of the global atmospheric circulation on Venus.

Top-to-Bottom Action

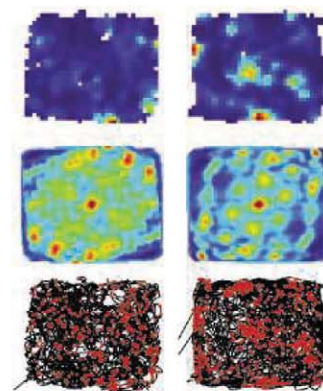
Hydrothermal vents supply large amounts of heat and chemical components to the deep ocean and support unique communities of organisms. Winds transfer great quantities of energy to the ocean and can help create eddies that extend far beneath the surface. **Adams *et al.*** (p. 580) suggest that these eddies can influence deep ocean flow below 2 kilometers and can help transfer heat and chemicals from deep ocean vents, as well as dispersing larval stages of organisms. Time-series observations of larval organisms, currents, and chemical and mass fluxes near the East Pacific Rise indicate that transport is associated with the passage of mesoscale eddies above. Interactions between surface winds and deep-sea currents may thus enable long-distance dispersal of larvae to supply isolated, ephemeral habitats and communities of organisms and provide a seasonal component to deep-sea processes.

No Fusion Confusion

Placental syncytins and *Caenorhabditis elegans* fusion family (FF) proteins, EFF-1 and AFF-1, can mediate eukaryotic cell-to-cell fusion. EFF-1 must be expressed in both fusing cells and must act via homotypic interactions. This fusion process has a hemifusion intermediate resembling viral and intracellular membrane fusion processes. Now, **Avinoam *et al.*** (p. 589, published online 24 March) show that FF homologs, from the human parasitic nematode *Trichinella spiralis* and the chordate *Branchiostoma floridae*, induce fusion in mammalian cells, indicating that the FFs are a family of conserved fusogens. Furthermore, AFF-1 can complement a fusion-deficient enveloped virus, rescuing its fusion defect and altering its infection mechanism.

Disrupting the Grid

Grid cells are neurons in the medial entorhinal cortex that have multiple firing peaks that organize into a highly regular hexagonal pattern in two-dimensional space. **Koenig *et al.*** (p. 592) and **Brandon *et al.*** (p. 595) used different drugs to inhibit neuronal firing in the medial septum (the “pleasure zone”), which caused a reduction of theta rhythm oscillations and prevented the grid cells from firing. However, cells that sense head-direction and cells with mixed grid and head direction properties continued to fire appropriately, and the cells in the hippocampus that sense location, the place cells, were not disrupted either. Thus, input from the medial septum is not necessary for all aspects of spatial processing.



Signal Stealer

Cytotoxic T lymphocyte antigen 4 (CTLA-4) is expressed by activated and regulatory T cells and helps to prevent T cell–mediated immune responses from getting out of control. The costimulatory pair of ligands bind CTLA-4 and are expressed on antigen-presenting cells. CD80 and CD86 also bind to CD28 expressed on T cells, which is critical for T cell activation. **Qureshi *et al.*** (p. 600, published online 7 April; see the Perspective by **Sakaguchi and Wing**) find that CTLA-4, expressed by both human and mouse T cells, plucks CD80 and CD86 from the surface of antigen-presenting cells and internalizes them through trans-endocytosis. These findings suggest an extrinsic cell mechanism for the negative regulation of T cell immunity by CTLA-4.

CREDIT: BRANDON ET AL.



Bruce Alberts is Editor-in-Chief of *Science*.

The New Egypt

I HAVE JUST RETURNED FROM EGYPT, WHERE I ATTENDED THE ANNUAL BOARD MEETING of the Library of Alexandria and met with students and faculty at The American University in Cairo. This is a very exciting time to be in Egypt, with its people empowered by the success of their daring, peaceful demonstrations in Tahrir Square. But the exhilarating sense of freedom is combined with the tension of knowing that the revolution is still in progress and its end point not yet known. Clearly, a great deal of hard work will be needed to establish an effective democracy. In media interviews, I was repeatedly asked, “What should the role of science be in the new Egypt?”

Science is a globally generated, ever-increasing base of sophisticated knowledge about the natural world that greatly benefits humanity. The benefits include labor-saving devices, improved health and nutrition, and many other advances that increase a nation’s prosperity and keep its voters satisfied. But only nations with strong, science-based institutions can effectively harvest this invaluable global source of knowledge, extending and adapting it to meet national needs. Democracies also require the creativity, rationality, openness, tolerance, and respect for evidence and logic that are inherent to science. The prime minister of India, Jawaharlal Nehru, recognized this 60 years ago when he called for a “scientific temper” for his newly independent nation, and a scientific temper is critical to every thriving democracy.

But as my visit made clear, a third aspect of science holds a special relevance for the new Egypt. Scientists in general take it for granted that, to be successful, the scientific enterprise must operate as a meritocracy. Ideas, results, and opinions must be evaluated independently of their sources, because it is what is said that is important, not who says it. Fitting with the democratic spirit of the Egyptian revolution, the scientific results of a young scientist are inherently no less deserving of respect than those of a senior Nobel Prize winner. For a nation to excel in science, college faculty, university leaders, and those who receive funding for research projects must be selected through competitive mechanisms that are entirely based on merit. Likewise, it is the establishment of a strong merit-based culture in both the public and the private sectors that will make the new Egypt successful.

There are lessons to be learned from the mistakes made by other nations. For example, years ago I was shocked by the remarks made by a scientific leader in India who, when asked about the major problem in his large research institute, told me that it was “getting people to work.” In that conversation, I discovered that in India’s government institutions, life tenure in one’s position is normally granted after 1 year of work. This has also been the case in Egypt, and it has contributed to the widely recognized nonperformance of the Egyptian government. I do not believe that it is possible to create an outstanding organization—be it a division of government or a university—with such demoralizing rules. Clearly, an institution thrives when its individuals are not only held accountable for their work, but also when each person is judged by his or her merits, without respect to the individual’s social status or personal connections.

But here is the problem. Who judges the merit, deciding which employees should be promoted and who removed? In science, unbiased peer review provides the foundation on which merit is decided. A similar type of peer review is generally used throughout higher education systems in the United States to produce excellence. But my nation is in the midst of a vigorous debate about how to shift to a more merit-based system for rewarding the 3 million teachers in our public school systems, where a lack of trust in leadership has long prevailed. For the new Egypt, as for the United States, finding a way to ensure that all institutions are merit-based will be a difficult, but absolutely critical, task.

— Bruce Alberts



HYDROLOGY

A Watershed Moment

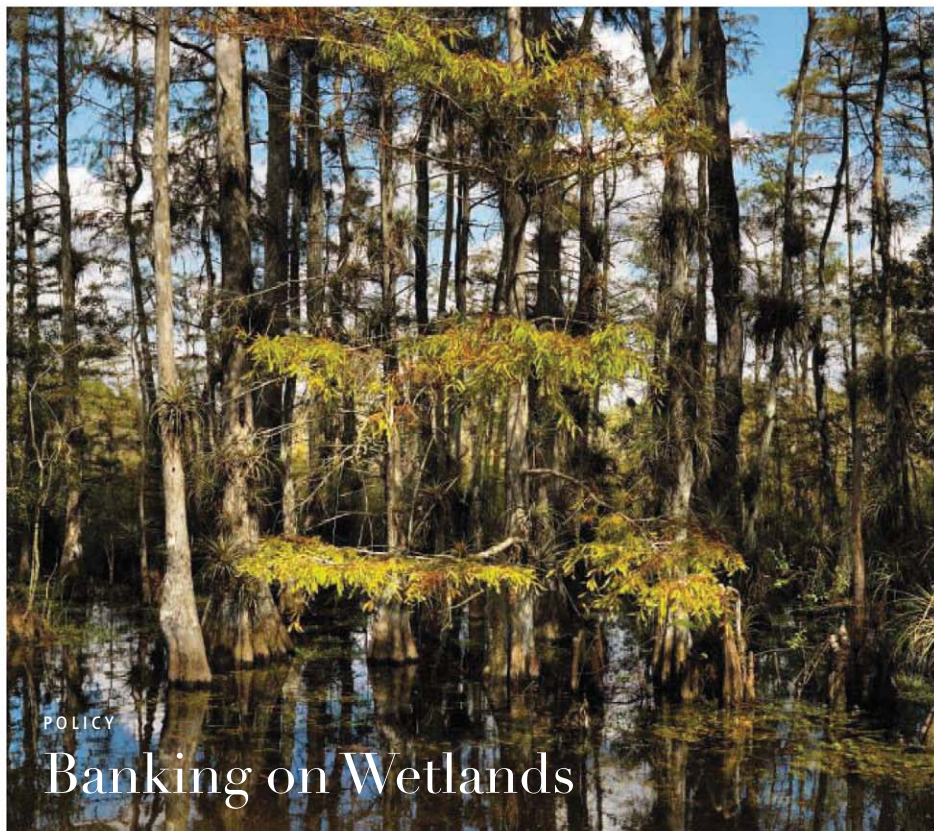
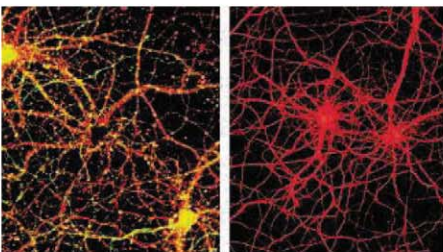
With increasing population and climate change exerting pressures on water resources, efforts to improve water quality are under way across a range of governmental levels. Yet water management strategies such as setting aside land for conservation can be highly variable between jurisdictions, and often watersheds cross national or international borders, making assessment of such efforts difficult. Wickham *et al.* catalogued and analyzed over 5000 drinking water watersheds in the conterminous United States, documenting some of the most important land-use factors controlling water quality, such as natural vegetation ground cover and extent of urbanization. From 1992 to 2001, about five times more watersheds showed a sizable decrease in natural vegetation cover than showed a comparable increase. Urbanization increased in 75% of the watersheds—9% showing more than a 1% increase. Because only a small fraction, particularly in the eastern United States, is conserved land, the growth of urbanization may soon exceed new conservation efforts. — NW

Landscape Ecol. 10.1007/s10980-011-9591-5 (2011).

NEUROSCIENCE

Soluble A β Is Trouble, Too

Cognitive impairments from Alzheimer's disease cause considerable loss of independence among the elderly. Insoluble aggregates of amyloid β -protein (A β) characterize brains from persons afflicted with Alzheimer's disease. Accumulating evidence suggests, however, that the neurotoxicity is actually due to the soluble forms of A β . Jin *et al.* have now analyzed one particular soluble form, the A β dimer, and its effects on neurons. A β dimers isolated from human brain tissue induced degeneration and cytoskeletal disruption in cultured neurons derived from embryonic rat hippocampi. A β monomers were not as effective. Antibodies to soluble A β diminished the toxic effects on neurons, lending support to an immunotherapy strategy currently in clinical trials. Soluble A β mediated its effects at least in part through tau, which drives the formation of the



A regulatory strategy adopted in many countries to promote ecosystem health (such as wetlands preservation) establishes markets in which permits issued to parties who damage ecosystems are traded for credits earned by parties whose projects mitigate damage in other ecosystems. In the United States, such mitigation efforts are undertaken by government agencies that use fees collected from permittees [in lieu fees (ILFs)]; by permittees themselves [permittee responsible mitigation (PRM)]; or by mitigation banks, private firms that conduct mitigation projects to earn credits that can be sold at market. In 2008, recognizing private banks as the lowest-risk option of these three approaches, the United States issued regulations intended to encourage banking growth. A survey of 156 mitigation professionals by BenDor and Riggsbee, however, shows that many perceived barriers persist. Although regulations were intended to streamline approval of projects, 44% of respondents reported no change in timelines. Only 48% of respondents felt that the regulations-established hierarchy of preferring banks over ILFs and PRM was actually applied. Moreover, 75% did not believe that financial risk had been reduced. The authors argue that many of these problems could be addressed by making regulatory policy-making more centralized. This could overcome what respondents perceive as excessive uncertainty resulting from fragmented regulation across several dozen jurisdictions. — BW

Environ. Sci. Policy 14, 301 (2011).

neurofibrillary tangles that are also a hallmark of Alzheimer's disease pathology. — PJH
Proc. Natl. Acad. Sci. U.S.A. 108, 5819, (2011).

CELL BIOLOGY

Don't Eat Me

Mitochondria, the power plants of the cell, come in a stunning variety of shapes and sizes. One reason for this is that depending on the physiological state of the cells, mitochondria can fuse

with one another and then separate from one another in an ongoing process of fusion and fission. When cells are deprived of nutrients, they will often induce autophagy—a process that allows for the degradation of bulk cytosol and whole organelles, including mitochondria, to provide raw materials to maintain essential cellular activities. Gomes *et al.* wanted to understand how and whether the dynamic state of mitochondria is affected by, and in turn affects, autophagy. When autophagy was induced, mitochondria were

observed to elongate, because mitochondrial fission was inhibited by the phosphorylation of a pro-fission protein, dynamin-related protein 1. These elongated mitochondria were able to resist autophagy and could maintain cell energetics despite nutrient deprivation. Conversely, blocking mitochondrial fusion precipitated starvation-induced cell death. — SMH

Nat. Cell Biol. **13**, 10.1038/ncb2220 (2011).

BIOMEDICINE

Exosomes Deliver

Exosomes are small vesicles secreted from cells that can carry proteins and nucleic acids between cells. Alvarez-Erviti *et al.* have exploited this process to deliver specific RNAs to target cells that lie across a major obstacle: the mammalian blood-brain barrier. Exosomes were isolated from mouse dendritic cells. In order to ensure targeting to the brain, the protein Lamb2b, which is normally expressed by dendritic cells, was tagged with a sequence from rabies viral glycoprotein that binds to a receptor expressed in brain tissue. The exosomes were loaded with small interfering RNA that targets BACE1, a protease that processes β -amyloid protein and produces the aggregates associated with Alzheimer's disease. When injected intravenously into mice, the exosomes crossed the blood-brain barrier and were taken up by brain neurons, oligodendrocytes, and glial cells, thereby decreasing the expression of BACE1 by approximately 60%. Nonspecific uptake of exosomes by other tissues was not observed, nor did the exosomes elicit an adverse immune response. The study suggests that targeted exosomes may have potential value for delivering therapeutic agents into the central nervous system to treat neurodegenerative disorders. — LC

Nat. Biotechnol. **29**, 341 (2011).

CHEMISTRY

Look Out for Traps

The intricate glassware notwithstanding, chemistry at the molecular level has traditionally been a passive activity. Trillions upon trillions of molecules are mixed together somewhat crudely, and then they are left to their own devices in the (often informed) hope that they will collide with one another in just the right way to rearrange into a desired product. With the increasing sophistication of laser technology, however, schemes have been proposed over the past several decades to manipulate the process more actively. The idea is to tailor a laser pulse in fine enough detail to steer atoms or molecules precisely along a landscape of quantum-mechanical energy states, and thereby to direct their behavior. And what should such a control pulse

look like? To find out, it's often simplest to try out a few at the outset, and then keep tuning the most effective ones iteratively until the optimal outcome is attained. Analysis of this approach suggested that, if there were an optimum pulse, the iterations would proceed cleanly toward it, without becoming trapped around a local maximum in the ensemble of possible pulses. Pechen and Tannor now show mathematically that the situation is more complicated. They draw a distinction between kinematic and dynamic critical points, dealing respectively with the time evolution operator of the system subjected to the control parameter and the control parameter itself. Even when kinematic traps are absent, second-order dynamic traps can arise, as the authors demonstrate using a three-level model system. — JSY

Phys. Rev. Lett. **106**, 120402 (2011).

CLIMATE SCIENCE

Greenland's Going Rate

One of the most potentially important consequences of global warming is sea-level rise. The Greenland Ice Sheet is expected to be the source of much of the meltwater that raises sea level in the near term, but how much mass loss it will experience in a warmer future is difficult to say with confidence, due largely to the difficulty of modeling the dynamic behavior of the ice-sheet as air and sea temperatures rise. Ren *et al.* present results from a multiphase, multiple-rheology, scalable and extensible geofluid ice-sheet model that has been designed specifically with that problem in mind. Their model incorporates full Navier-Stokes equations to account for nonlocal



dynamic balance and ice flow, and a granular sliding layer between the ice and bedrock to allow large-scale surges like those that are commonly observed now. Forcing their model with monthly atmospheric conditions provided by high-resolution climate simulations, they project that the rate of Greenland Ice Sheet mass loss could reach as high as 220 km³/year by 2100, significantly exceeding estimates by the IPCC AR4 of ~50 to 100 km³/year, which were made without considering the dynamic behavior of the ice sheet. — HJS

J. Clim. **10.1175/2011JCLI3708.1** (2011).



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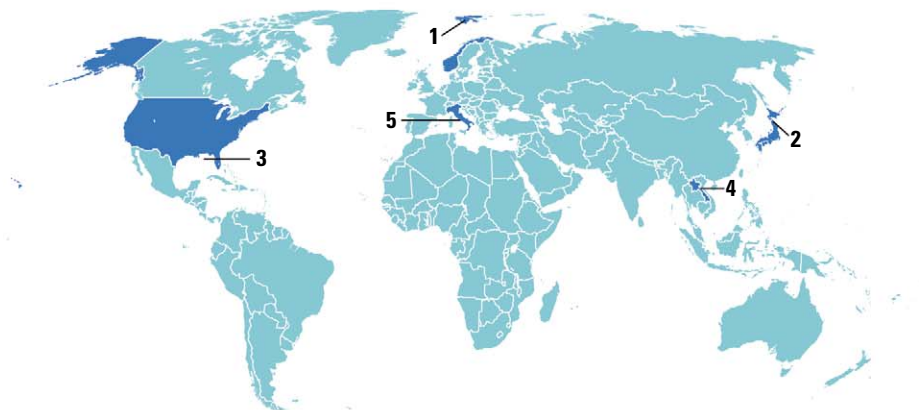
* For the purpose of this prize, molecular biology is defined as "that part of biology which attempts to interpret biological events in terms of the physico-chemical properties of molecules in a cell".

(*McGraw-Hill Dictionary of Scientific and Technical Terms*, 4th Edition).

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AROUND THE WORLD



Svalbard, Norway 1

Exploring the Darkening Arctic

Thirty scientists from the United States, Norway, Germany, Italy, Russia, and China have completed the first phase of a 2-month expedition to understand the origin and effects of soot on the Arctic's changing climate. Modeling suggests that, by darkening the usually white landscape, soot could be responsible for as much as 75% of warming there.

From an outpost on the Svalbard archipelago off Norway, the team is deploying remotely operated aircraft to take air samples and solar radiation measurements. The first phase tracked soot's effects over land; now the drones are exploring sea ice. But unexpected movements of ice—perhaps due to warming or winds—have forced the scientists ground-truthing the data to change the areas they target. “Normally the fjord has melted a lot less than it did this year,” says team member Timothy Bates, an atmospheric chemist with the U.S. National Oceanic and Atmospheric Administration. “The snowmobiles that we use for the ground truthing can no longer get to these sites.” They plan to present results by December.

Fukushima, Japan 2

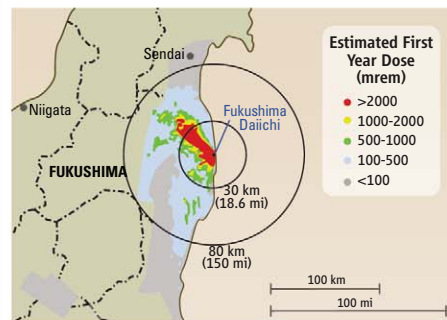
U.S. Scientists Map First-Year Radiation Risks

The U.S. Department of Energy (DOE) has sketched out the radiation risks over the next year to people living near Japan's ailing Fukushima Daiichi nuclear plant.

DOE-sponsored aerial surveys began measuring radiation around the plant about 1 week after the reactor was hit by a tsunami on 11 March. Now DOE has projected the first-year radiation dose within 80 kilometers. A map released last week (pictured)

shows a hot zone out to 50 kilometers northwest of the plant where weather deposited a lot of fallout. There, potential exposures exceed 2000 millirems per year, the level at which the U.S. Department of Homeland Security would consider relocating the public. Japan has already advised that residents leave some towns outside the 20-kilometer evacuation zone.

Although 2000 millirems over 1 year isn't an immediate health threat, it's enough to cause roughly one extra cancer case in



500 young adults, says Owen Hoffman, a radiation risk expert with SENES Oak Ridge Inc. in Tennessee. Still, there are parts of the world where natural background radiation levels are even higher.

<http://scim.ag/future-rad>

Gulf of Mexico 3

Scientists Look to Gulf's Future

One year since oil began gushing into the Gulf of Mexico from BP's Macondo well, officials have reached key milestones for boosting science and the environment.

On 25 April, the Gulf of Mexico Research Initiative (GRI) announced its first request for proposals (RFP). BP set up GRI to provide \$500 million over 10 years for independent research on topics from ecological



impacts to cleanup technology. Scientists have been waiting for the RFP since BP distributed an initial \$50 million last year. GRI's board of scientists plans to fund between four and eight collaborations, but won't start until late August, leaving some scientists concerned about a funding gap.

Meanwhile, BP has agreed to pay \$1 billion for restoration projects, to be selected by the five gulf states and two federal agencies. A down payment on the eventual settlement under the government's Natural Resource Damage Assessment, the money will jumpstart activities such as rebuilding wetlands. “You need to begin these things sooner rather than later,” says Donald Boesch, a marine biologist at the University of Maryland Center for Environmental Science in Cambridge. The proposals will be available for public comment and subject to federal environmental review, but not scientific vetting—and that's unfortunate, Boesch says. <http://scim.ag/bp-funds>

Lower Mekong River, Laos 4

Planned Dam Put on Hold

Citing environmental concerns, Southeast Asian nations last week halted plans for a 1260 megawatt hydropower dam on the Mekong River in northern Laos. “It's a real victory for science,” says Zeb Hogan, a fisheries biologist at the University of Nevada, Reno.

The \$3.8 billion Xayaburi dam would be the first of 11 dams planned for the Lower Mekong. Proponents say the sale of electricity, primarily to Thailand, would boost Laos's economy and that environmental consequences would be mitigated. But opponents worry that the dam would affect the world's most productive fishery, in which migratory fish are 40% to 70% of the catch. “As it's currently conceived, the new dam could have disastrous effects on biodiversity,” asserts Hogan, director of the National Geographic Society Megafishes Project. It would impede migration, he says, and possibly drive to extinction the critically endangered Mekong giant catfish and the dog-eating catfish, both of which can reach 3 meters in length.

At a 19 April meeting in Vientiane of the Mekong River Commission, Vietnam

called for a 10-year moratorium on hydro-power projects on the mainstream Lower Mekong. Ministers from the four commission members—Cambodia, Laos, Thailand, and Vietnam—will meet again in October to reconsider the project.

Rome 5

Nuclear Power Stalls in Italy

Plans to build new nuclear reactors in Italy have stalled following March's accident at the Fukushima plant in Japan.

In 2008, Italy's government announced it wanted to construct four nuclear plants by 2013 to reduce its dependence on imported energy. That would reverse a ban on nuclear energy set after the 1986 Chernobyl disaster. But the Japanese accident led Italy to put a 1-year moratorium on its nuclear program, and, last week, to delay the creation of new plants indefinitely.

Davide Tabarelli, head of Italian energy consultants Nomisma Energia, says the move is no surprise. He points out that the nuclear program has encountered delays and opposition from regional governments unwilling to host new plants. Opposition politicians are accusing the government of introducing the moratorium to avoid a June referendum on the return of nuclear power that it looked set to lose.

Economic minister Paolo Romani said the government will present a new energy strategy after the summer. Such a plan is long overdue, Tabarelli says, but he is skeptical it will accomplish Italy's renewable energy goals. <http://scim.ag/italy-nuclear>

NEWSMAKERS

Three Q's

In the Chauvet Cave system in southern France, a prehistoric art gallery holds stunning paintings of animals and humans dating from 32,000 years ago (*Science*, 15 August 2008, p. 904). At one point the entrance caved in, preserving the paintings until their discovery in 1994. German filmmaker **Werner Herzog** gained exclusive access to the cave. His 3D documentary, *Cave of Forgotten Dreams*, which opens today in theaters, tours the cave and chronicles scientists' efforts to unearth its secrets.



Q: Why was it important to film this cave?

It's a totally unique discovery, possibly one of the greatest discoveries in the history of human culture. ... What makes it so extraordinary is the unbelievable quality of the paintings and the age of them. [In getting permission to film, I found that] the French can be territorial when it comes to "le patrimoine," but I explained that this is the patrimony of the entire human race.

Q: What was it like when you stepped into the cave?

I was totally surprised by the beauty of the cave: stalactites and stalagmites, crystal cathedrals, that's the first you see. Then, all of a sudden, bones, bones, bones: skeleton remains, cave bear, hyena, the full skeleton of a golden eagle, the footprints of a wolf. ...

What you can't really transport into the movie is the amount of silence. When you hold your breath, you can hear your heartbeat.

Q: What surprised you about the paintings and their painters?

How fresh they are, that's stunning. As if you were disturbing people right in the middle of their work. ... The most intense is the panel of lions. They're in there stalking. Every single eye is focused on one point and you don't know: What do they see? What do they stalk? It has such suspense, such drama.

FINDINGS

Don't Pet That Armadillo

Armadillos are familiar creatures in the American southwest, where they haunt gardens and roadsides. But scientists have long eyed the animals with distrust, considering them suspect number one in transmitting leprosy to people in the United States. Now, that suspicion seems justified.

This week, a team of top leprosy experts reports in *The New England Journal of Medicine* that they've found a particular strain of *Mycobacterium leprae*, which causes the skin and nerve disease, in a wild armadillo and three U.S. patients. Genetic sequencing revealed that it is a new strain that appears to be indigenous to the United States—it hasn't been

described anywhere else in the world. Further testing uncovered the



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Jurassic Spider Not So Itsy Bitsy

A new fossil discovered in the Daohugou Beds of north-eastern China shows that a modern group of spiders, called golden orb-weavers or *Nephila*, were on Earth during the Jurassic period about 165 million years ago. The fossil, *Nephila jurassica*, is a female, and if modern spiders are any guide, was probably much larger than her male counterpart. With a 15-centimeter-plus leg span, she's the largest fossilized spider ever found, lead author Paul Selden of the University of Kansas, Lawrence, and colleagues report online in *Biology Letters*. She appears to be closely related to the modern web-weaving *Nephila* spiders of the same size. Well-preserved spinnerets on the fossil's abdomen suggest that it, too, spun large, permanent webs: a weapon in an arms race that could have influenced the evolution of insects and small birds that got caught in them.



BY THE NUMBERS

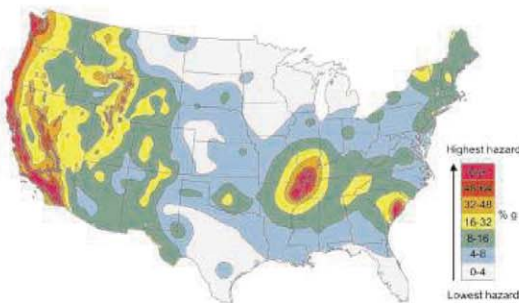
4.67×10^{32} The intensity, in protons per square centimeter per second, of the Large Hadron Collider's intersecting proton beams as of last week. That "luminosity" surpasses the Tevatron's record.

\$56 million Amount that Detroit developer A. Alfred Taubman donated last week to the University of Michigan to fund stem cell research, the school's largest gift. Taubman has donated a total of \$142 million to the university.

>>FINDINGS

same strain in 28 of 33 wild armadillos and 25 of 39 U.S. leprosy patients. The authors speculate that armadillos pass the bacteria efficiently to one another.

"All of a sudden it's everywhere," says John Abide, a dermatologist in rural Greenville, Michigan, who just 2 weeks ago came across his fourth suspected case of leprosy, in an older man. Armadillos might be cute, he says, "but you just got to stay away from them." <http://scim.ag/arma-dillo>



Central U.S. Quake Threat Real

Two hundred years ago this December, a series of three large earthquakes centered in southeastern Missouri destroyed the little town of New Madrid and rattled the entire eastern half of the country. Four similar quake clusters have struck the same area over the past few thousand years. But some seismologists have said the risk of another damaging New Madrid quake is now past.

Don't believe it, says an eight-member expert panel convened by the National Earthquake Prediction Evaluation Council. In its new report, the panel concludes that while

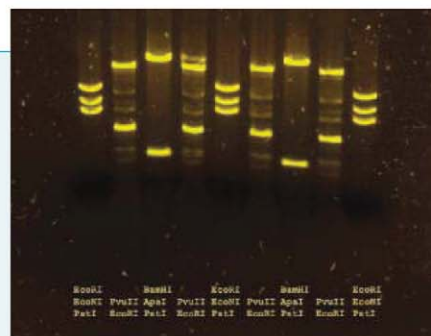
Random Sample

Art Imitating Life—
The Synthetic Kind

A handful of artists are taking on a truly avant-garde new medium: synthetic biology, the melding of engineering and biology to build living systems.

Only 17% of Europeans have even heard of synthetic biology, according to a 2010 study conducted for the European Commission, yet its implications for the future are enormous. So to get the public more involved, biosafety scientist Markus Schmidt has organized Bio:Fiction, the world's first synthetic biology film festival, to be held at the Museum of Natural History in Vienna from 13 to 14 May. Bio:Fiction will feature 52 films—130 were submitted—ranging in length from 59 seconds to 59 minutes and running the gamut from explanatory nonfiction to abstract works. (One is a love story about protocells.) Five films, including the one that gets the most online votes (<http://bio-fiction.com/videos/>), will share \$13,000 in prize money.

An accompanying art show, *Synth-ethic*, will have a lablike feel. One piece is a replica of a famous experiment on the origin of life, complete with periodic lightning bolts inside a flask. Another uses electrical currents to arrange gel-bound DNA fragments into images (top). A pigeon-feeder installed in an open window represents an art-science collaboration's efforts to engineer soap-producing bacteria for pigeon feed: Hello, clean windshields. "More and more artists are appropriating the tools of biology to express themselves," Schmidt says.



it's conceivable that the New Madrid threat is past, "we and most other experts consider this claim unlikely."

But forecasting quakes in this area is difficult, largely because scientists don't understand the drivers. The motions of tectonic plates, which cause quakes around the Pacific and at other plate edges, don't explain quakes in the interiors of continents. And there are precious few geophysical signs that New Madrid could be building toward another large quake. Given all the uncertainty, says the panel's chair, seismologist John Vidale of the University of Washington, Seattle, "the prudent thing to do is assume earthquakes will continue." <http://scim.ag/new-madrid>

Spreading Disease-Proof
Mosquitoes

Suppose you have designed a mosquito that can't transmit malaria or another major scourge. Now what? If you release the critters, they will be vastly outnumbered by

the billions of natural mosquitoes already out there. Now, scientists have developed a genetic trick that could help disease-resistant mosquitoes spread like wildfire.

A team led by molecular biologists Andrea Crisanti and Austin Burt of Imperial College London reported online in *Nature* last week the development of a so-called gene drive system. It uses a homing-endonuclease gene (HEG), which creates a second copy of itself in individuals that have only one, ensuring that all offspring have the gene.

The researchers bred a population of *Anopheles gambiae* mosquitoes that glow in the dark, thanks to a gene that produces green fluorescent protein (GFP). Then they released into the insects' cages small numbers of mosquitoes with a HEG that breaks up the GFP gene in sperm cells and swaps itself in on the chromosome. Over generations, fewer mosquitoes were glowing, indicating that the gene spread rapidly. The next step, Crisanti says, is to make HEG break up a mosquito gene crucial for malaria transmission. <http://scim.ag/disease-proof>

Different orbits? Ray Arvidson teaches students at Shandong University about the geological evolution of Mars.



INTERNATIONAL COOPERATION

U.S. Spending Bill Limits Joint Efforts With China

Representative Frank Wolf (R-VA) is a fierce critic of the Chinese government. He also chairs a congressional spending panel that oversees the budgets of NASA and several other federal research agencies. This month, he used his clout to add a spending ban to the final agreement on the 2011 budget that cuts off funding for a host of scientific interactions between the United States and China.

The two sentences Wolf inserted into the bill that averted a government shutdown (*Science*, 15 April, p. 291; 22 April, p. 407) prohibit any joint scientific activity between the two nations involving NASA or the White House Office of Science and Technology Policy (OSTP). White House and NASA officials say they are still reviewing the language, which on the surface appears to apply only to those two entities and extends only for the rest of the 2011 fiscal year, which ends on 30 September. But that still cuts a wide swath. In January, OSTP Director John Holdren and Chinese Science Minister Wan Gang signed an extension of a 1979 agreement on science and technology cooperation; a fact sheet cites cooperative research between U.S. agencies and their Chinese counterparts covering “fisheries, earth and atmospheric sciences, basic research in physics and chemistry, energy, agriculture, civil industrial technology, geology, health, and disaster relief.”

Last week, Wolf told *Science* that he intends the prohibition to apply to all projects

covered by the newly renewed agreement, adding that he would like to permanently shut down all collaborations between the two governments. “We don’t want to give [China] the opportunity to take advantage of our technology, and we have nothing to gain from dealing with them,” Wolf says. “And frankly, it boils down to a moral issue. ... Would you have a bilateral program with Stalin?”

Put that question to Ray Arvidson, a planetary geologist at Washington University in St. Louis (WUSTL), and his answer probably resonates with generations of scientists who have worked with colleagues from a country whose government is unlike, or even hostile to, their own. “It’s a complex world,” he acknowledges. “But ignoring all that [China] is doing in space is not the right approach, in my opinion.”

Arvidson helps manage an archive that collects and distributes information from NASA’s planetary missions—past, present, and future. He has spent the past 5 years training space scientists at Shandong University in southeastern China to use the Planetary Data System (PDS). As China’s space program expands, Arvidson hopes its scientists will overcome their historical reluctance to share data and use PDS to archive their results or to create a compatible system that the rest of the world can access. He’s eager to see if his efforts will bear fruit in China’s first planetary mission, Yinghuo 1, an orbiter to be launched

in December as part of a Russian mission to Mars, dubbed Phobos Grunt.

Arvidson’s work on the PDS geosciences node would not be covered by the new language: It is funded by the endowment at WUSTL, a private institution, and the agreement is between the two universities, not their governments. But Arvidson says, “I’m looking forward to the day when we can submit proposals to NASA to foster this collaboration.” That seems unlikely to happen anytime soon, however, given Wolf’s language and the sensitive nature of dealing with China on any space-related issue.

NASA’s Ed Grayzeck, project manager for PDS, emphasizes that NASA “has no formal agreement with China” regarding the upcoming Mars launch or any other planetary mission. But he says Arvidson and Ray Walker, a geophysicist at the University of California, Los Angeles, who maintains a second PDS node on plasma physics, have developed a template for how such collaborations can nevertheless move ahead. Other spacefaring countries have created similar archives, he notes, and 3 years ago the nongovernmental organization International Planetary Data Alliance was formed to facilitate the global sharing of such information. Although NASA officials are still reviewing the language in the 2011 spending bill, Grayzeck says that his initial impression is that it “is extremely restrictive.”

That is his intent, says Wolf, who has long criticized China’s suppression of religious and minority groups and political dissidents, as well as its policies toward Tibet. He’s also used his position as chair of the House of Representatives spending panel on commerce, justice, and science to urge federal agencies to beef up their defenses against cyberterrorism, much of which Wolf believes originates in China. At one recent hearing, for example, Wolf grilled the National Science Foundation’s (NSF’s) inspector general about a December 2010 attack on NSF computers that, ironically, affected grant applications to its Office of Cyberinfrastructure.

Wolf says his rider is meant to deter the Obama Administration from expanding its relationship with China. “China is spying against us, and every U.S. government agency has been hit by cyberattacks,” Wolf says. “They are stealing technology from every major U.S. company. They have taken technology from NASA, and they have hit the NSF computers. ... You name the company, and the Chinese are trying to get its secrets.”

—JEFFREY MERVIS

ASTRONOMY

Peering Back 13 Billion Years, Through a Gravitational Lens

In 1979, astronomers reported the first example of gravity's ability to bend light when they imaged two identical quasars close to one another. The twin images were in fact a single object; the gravity of a galaxy cluster sitting between the quasar and Earth had bent the light to produce a double image. Since then, astronomers have exploited this effect, known as gravitational lensing, to study sources of light that otherwise would have been too faint to see and also estimate the mass of galaxy clusters causing the lensing. These studies have helped researchers discover distant galaxies, find exoplanets, and study the structure of the universe. Now, a team of researchers led by Sudeep Das at the University of California, Berkeley, has made the first direct detection of gravitational lensing of cosmic microwave background (CMB) radiation, the faint afterglow of the big bang that suffuses the universe.

Das and his colleagues have observed how the leftover radiation from the big bang has been deflected by gravity along its journey over 13 billion years from the primordial to the present-day universe. Besides confirming that gravitational lensing affects the CMB just as it affects starlight, the result paints a broad-brush picture of how matter is distributed along the radiation's pathway, which verifies theorists' favorite model of

how the structure of the universe has evolved since the big bang. The researchers say that more precise measurements of CMB lensing in the future could help determine the mass of neutrinos.

The detection is a "milestone," says Charles Bennett, an astrophysicist at Johns Hopkins University in Baltimore, Maryland, who was not involved in the research. The work "represents a successful independent test of a prediction made by the laws of physics and the standard model of cosmology," Bennett says. Lyman Page, a Princeton University astrophysicist who collaborated on the project, says, "We are pretty happy with the result and think it foreshadows a rich new vein in cosmology." The researchers report their findings in a paper submitted to *Physical Review Letters* and posted on arXiv.org last month.

The CMB is like a backlight to the cosmos that appears nearly the same wherever you look in the universe. However, there are small differences in the temperature of the radiation between different points in space, which can be traced back to tiny fluctuations in the density of the universe when it was a hot ball of hydrogen 380,000 years after the big bang. Over the past decade, the Wilkinson Microwave Anisotropy Probe (WMAP), a spacecraft launched in 2001, has enabled researchers to map the CMB by measuring these temperature variations.

To search for small distortions that might have been caused by lensing,

Das and his colleagues used the Atacama Cosmology Telescope, a 6-meter instrument in northern Chile that measures the temperature differences in the CMB at 20 times the resolution afforded by WMAP. They compared the telescope's fine-grained picture of the CMB for a patch of sky with a simulation of what the CMB would look like if it had been lensed.

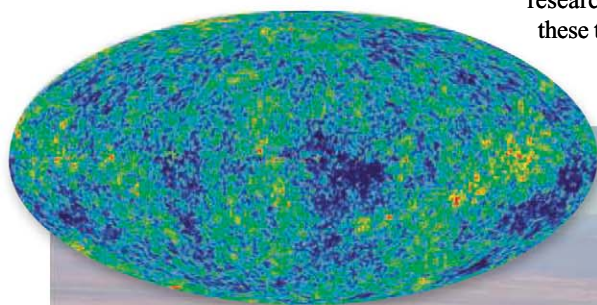
For the simulation, the researchers used what's known as the lambda cold dark matter model of the universe, which predicts how cosmic structure has evolved since the big bang. The observations closely matched the simulated picture. "It was mind-boggling," Das says.

Other groups had previously detected CMB lensing indirectly, by uncovering distortions in the CMB in regions that harbor known galaxies, where there was likely to be large amounts of dark matter exerting a strong gravitational pull. What Das's group has achieved "is technically a much harder thing to do," says astrophysicist Christopher Hirata of the California Institute of Technology in Pasadena, who led one of those earlier detections. A big advantage of the approach, he adds, is that it can detect lensing caused by very distant galaxies, at redshifts beyond the sight of the most powerful telescopes. "Hopefully in the future, the method will help study how structures were forming in very early epochs of the universe," Hirata says.

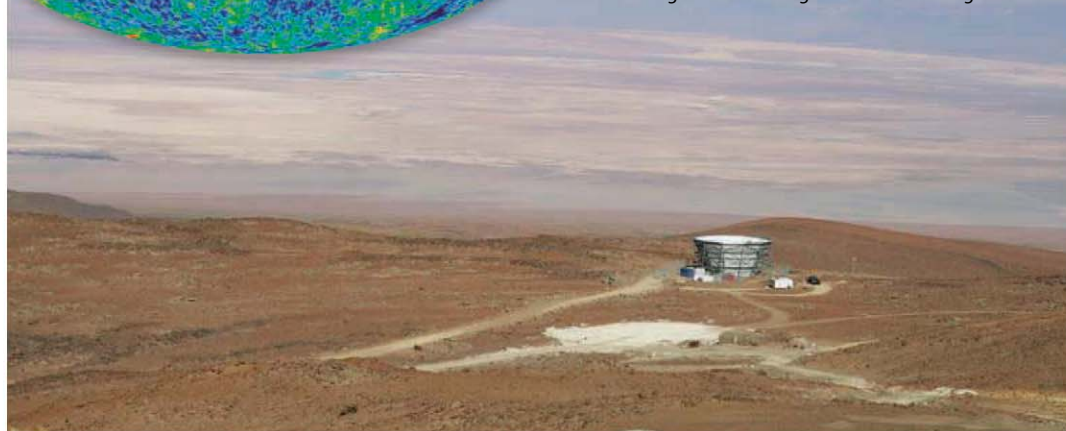
The technique could also help researchers determine the mass of neutrinos: elusive particles that zip around at velocities close to that of light and compose a portion of dark matter. Because they move so quickly, neutrinos are expected to have had a dispersive effect on the clumping of dark matter, preventing it from aggregating on small scales in the early universe. As a result, neutrino mass has a bearing on cosmic structure.

Measured with sufficient precision, the lensing of the CMB should allow researchers to test cosmological models using different neutrino masses, ultimately helping to zero in on the mass of the particle. "The sensitivity of CMB measurements is getting to the point that we should be able to have an observation of neutrino mass in the next generation of experiments," says Kathryn Zurek, a particle physicist at the University of Michigan, Ann Arbor.

—YUDHIJIT BHATTACHARJEE



High definition. The Atacama Cosmology Telescope has enabled astronomers to study the cosmic microwave background (*inset*) in fine detail, revealing the effects of gravitational lensing.



MEDICINE

Shortages of Cancer Drugs Put Patients, Trials at Risk

Two years ago when University of California, San Francisco, biochemist Thomas Kornberg was undergoing 6 months of grueling chemotherapy for Hodgkin lymphoma, his oncologist delivered bad news: UCSF's cancer center was out of one of the four drugs needed for his next round of treatment in 2 weeks. Kornberg called physician friends at area hospitals until one was able to find some of the drug, vincristine. Kornberg's cancer is now in remission. But he hasn't forgotten his panic at the time. "Going through [chemotherapy], one has a pretty helpless feeling to begin with, and additional complications just add to the anxiety," he says.

Kornberg's experience is not unusual. This month, a shortage of another cancer drug, cytarabine, a crucial drug for many leukemia patients, including children, drew an outcry from oncologists around the country who warned that hospitals were being forced to ration the drug and delay some patients' treatments or give them less effective therapies. "It's a nightmare," says Hagop Kantarjian, chair of the leukemia department at MD Anderson Cancer Center in Houston, Texas. Kantarjian penned a commentary in *The Washington Post* last week after querying 8000 colleagues about the shortage via newsletter and receiving "about 200 e-mails with horror stories," he says. By week's end, the crisis, which began last fall and worsened this year, eased as vials of cytarabine became available.

But the problem is far from solved; many other generic cancer drugs are in short supply, experts say. "This is the worst in 3 decades since it's been tracked," says oncologist Michael Link of Stanford University School of Medicine in Palo Alto, California, who is president-elect of the American Society of Clinical Oncology (ASCO). Shortages of other mostly generic drugs, including anesthesia drugs and antibiotics as well as cancer drugs, have tripled since 2006 to 211 shortages last year (see graph). With cancer, "the fear is that it could have an impact on survival," Link says. The drug shortage has drawn concern from National Cancer Institute (NCI) Director Harold Varmus, who recently asked for a briefing from his staff.

The 30 or so cancer drugs recently on shortage lists kept by the U.S. Food and Drug Administration (FDA) and others include cisplatin, busulfan, doxorubicin, etoposide, and leucovorin. Most are decades-old drugs that

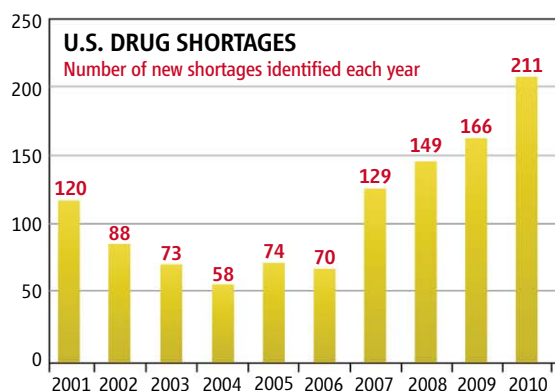
kill dividing cells and are highly toxic. But they are still mainstays in cancer treatment, including for certain cancers such as lymphoma and testicular cancer that are often curable—provided the right drugs are available.

The cytarabine shortage has been particularly worrisome because there is no substitute for treating acute myeloid leukemia, oncologists say. Supply problems loomed last year when an ingredient was in short supply, then worsened this year after two manufacturers, Hospira and APP Pharmaceuticals, found that some vials contained crystals, which made the product unsafe. A third company, Bedford Laboratories, could not meet the demand on its own. Hospira has since resolved the crystallization problem and resumed shipments. FDA is also temporarily allowing cytarabine importation, says Valerie Jensen, associate director of the drug shortage program at FDA.

Shortages can also complicate clinical trials that use the old drugs as a control or in combination with an experimental drug, Link says. If a specific drug isn't available, clinicians may have to delay enrollment or give enrolled patients a different drug, departing from the trial protocol. This could have repercussions when trial results are analyzed, Link says: "How do you analyze those patients who only got part of the therapy?" There have been scattered reports that drug shortages have affected some trials, forcing trial leaders to modify protocols to allow for substitute treatments, for example.

Part of the problem, Jensen says, is that many of those on the shortage list are injected drugs, which are complex to manufacture, and a dwindling number of companies are making them. Others point out that nearly all the scarce drugs are generics, which aren't very profitable, so companies have little incentive to add backup capacity. "The root cause of this is financial," Kantarjian says. "It has almost never happened with brand-name drugs."

In search of solutions, ASCO, the American Society of Health-System Pharmacists (ASHP), the American Society of Anesthesiologists, and the nonprofit Institute for Safe Medication Practices held a "drug shortages summit" in Bethesda, Maryland, last November. Lawmakers are already responding to



Growing concern. Drug shortages are on the rise. They include cytarabine, a cancer drug used to treat this young leukemia patient in Cleveland.

one recommendation: A bill introduced in February by senators Amy Klobuchar (D-MN) and Robert Casey (D-PA) would require manufacturers to notify FDA when they have production problems or expect to stop making a drug. This would allow FDA to work with other companies to ramp up production. The bill's prospects this year are uncertain, but at least "it's the beginning of a discussion," says Joseph Hill, director of federal legislative affairs director for ASHP.

Other ideas from the summit: Congress could give generic drug companies tax breaks to produce the most critical drugs and to upgrade manufacturing lines. Or the U.S. government could create national stockpiles of critical drugs, as it now does with bioterrorism countermeasures and the pandemic flu vaccine.

In the meantime, NCI is looking into creating a reserve of key cancer drugs to help alleviate shortages, officials there say. Kornberg hopes something can be done. "With all the billions we spend to develop the few drugs that actually work," he says, "the fact that we don't have a reliable supply is ironic to say the least."

—JOCELYN KAISER

INDIA

Faculties Wither as Higher Education System Rapidly Expands

NEW DELHI—At the Indian Science Congress in January 2008, Indian Prime Minister Manmohan Singh announced that his nation would launch a “second wave” of education. “We will need an army of teachers, especially in the basic sciences and in the field of mathematics,” he said. “The time has come for action.”

At the time, India was rapidly expanding its higher education system. The country has opened more than 100 elite higher education institutions in the past 5 years and is now home to 467 universities (see table). In the headlong rush, student enrollment has risen more than 50% to 13.6 million. Meanwhile, almost a third of faculty positions at elite institutions—both new and long established—are vacant due to an acute shortage of trained teachers. The result is a dilution of standards, says P. V. Indiresan, former direc-

tion minister, Kapil Sibal, acknowledged the dire situation. “Germination of ideas takes place through the university system, and if the system is not effervescent, energetic, and creative, then India would be relegated to the knowledge of the past,” he said. But the solution remains elusive.

Lack of funding is not the problem. In the past 5 years, the government has invested \$11.6 billion in education, a whopping 14-fold increase over the previous 5 years. As impressive as that sounds, that figure—1% of India’s GDP—could rise even higher. The government has vowed to make education a cornerstone of development by increasing education spending to 6% of GDP over the next decade. In 2011, spending will rise 19% to \$3.3 billion.

All that money can’t buy good teaching, apparently. A 2010 government survey

dying.” To ease the teaching shortage, a new National Knowledge Network will combine classrooms over the next 2 years to share the teaching workload with “distance education,” linking 1500 universities through a dedicated fiber-optic Internet link.

India is also hoping that foreign universities will help raise the game of indigenous institutions by offering competition. The Indian Parliament is considering a proposal that would provide the legal framework for overseas institutions to set up shop in India. Parts of the bill have raised concerns about built-in barriers, including a provision that would ask foreign institutions to make a deposit of at least \$12 million to regulators before opening a campus in India. That has deterred potential suitors, including the Georgia Institute of Technology in Atlanta and the University of California, Berkeley.

In the meantime, India has taken steps to increase the talent pool further up the pipeline. One program getting high marks is Innovation in Science Pursuit for Inspired Research, a government initiative spearheaded by the science ministry that in the past 2 years has awarded scholarships to 350,000 outstanding high school science students. The grants are small—about \$125 per student—but the intention is to boost morale and keep students interested in science, says T. Ramasami, secretary of the Department of Science and Technology here. “We hope to catch them young and build a cadre of top-quality researchers,” he says.

Ramasami and others have faith that India’s education system can regain lost luster—and uncover hidden talents like Mylswamy Annadurai. An engineer from Coimbatore in southern India, Annadurai admits he had never left the small town before completing his postgraduate engineering education. He went on to become project director for the country’s first moon mission, Chandrayaan-1. Annadurai credits his university experience for “daring to dream big while not worrying about failure.”

India desperately needs more homegrown stars, says Singh’s science adviser, chemist C. N. R. Rao of the Jawaharlal Nehru Centre for Advanced Scientific Research in Bangalore, who hopes his council’s report last week will spur the government to prepare a road map for higher education. “There is so much hidden talent in India” that will blossom when it finds fertile ground, Rao says.

—PALLAVA BAGLA



INDIA’S EDUCATION BOOM

8	Indian Institutes of Technology
7	Indian Institutes of Management
30	Central Universities
3	Indian Institutes of Science, Education and Research
20	Indian Institutes of Information Technology
20	National Institutes of Technology, the neighborhood engineering colleges
14	Specialized universities with an emphasis on research called “Innovation Universities”

Boom and bust. Although more than 100 elite institutions have opened in the past 5 years, competition is fiercer than ever. Last year, only 3% of students, including these at a cram school in Kota, India, passed IIT’s entrance exam.

tor of the Indian Institute of Technology (IIT) in Chennai. “Expansion is politically popular,” he says, “but functionally disastrous.”

Indian leaders are aware they’re grappling with a crisis in higher education. In a report issued last week, the prime minister’s science advisory council decried a number of serious problems, including “declining quality and indifferent performance of institutions” and a system that puts a greater emphasis on entrance exams than on education. The University Grants Commission of India recently rated “68% of the universities and 91% of the colleges” as average or below average. “This hardly speaks well of quality and excellence in universities,” the report states. At a meeting of university vice-chancellors last month, India’s educa-

tion minister, Kapil Sibal, acknowledged the dire situation. “Germination of ideas takes place through the university system, and if the system is not effervescent, energetic, and creative, then India would be relegated to the knowledge of the past,” he said. But the solution remains elusive.

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All that money can’t buy good teaching, apparently. A 2010 government survey

of India’s top 22 universities reports that 3777 out of 11,085 faculty positions were unfilled. The University of Delhi needs to hire approximately 3000 teachers, says historian Nayanjot Lahiri, a former dean at the university. The prestigious IIT, which has been expanded to eight new sites, reports a similar one-third shortfall. At the new IITs, even retaining freshly recruited faculty members is a challenge partly because of working conditions: Several institutes are still operating out of ramshackle temporary buildings.

A major obstacle to recruitment is the failure to make teaching an attractive career, says Man Mohan Sharma, a chemist at the Institute of Chemical Technology in Mumbai. “Teaching is no longer a glamorous or paying profession,” he says. “Scholarly habits are

INFECTIOUS DISEASES

'Breakthrough' Deal on Flu Strains Has Modest Provisions

When exhausted negotiators in Geneva finally reached a deal about the global sharing of influenza viruses early in the morning on Saturday, 15 April, the World Health Organization (WHO) was quick to call it a "landmark agreement." WHO chief Margaret Chan hailed the 45-page document as "a very significant victory for public health."

But the most significant breakthrough may be that, after 4 years of complex and often contentious negotiations, there is a deal at all. The actual text of the agreement—which promises developing countries certain benefits in return for sharing their flu viruses with the world—contains mostly "soft" language that's not legally binding, says international law professor David Fidler of Indiana University Maurer School of Law in Bloomington. And although the agreement promises developing countries access to drugs and vaccines during the next pandemic, it can't prevent them from ending up with the short end of the stick.

The agreement was necessary to salvage an international system that has developed over 5 decades. Each country shares its influenza isolates with WHO's collaborating centers, a group of six specialized flu labs that closely monitor changes in the flu virus's genetics and behavior. In 2006, Indonesia defied that system for the first time by refusing to share samples from its H5N1 avian influenza outbreak unless it had guarantees that it would also share in any resulting products—primarily vaccines—should H5N1 trigger a pandemic. The H1N1 pandemic of 2009–10, in which the vaccine supply was quickly snapped up by rich countries, drove home the need for a "framework" deal, says WHO top flu scientist Keiji Fukuda.

The new agreement, expected to be approved by the World Health Assembly in May, says that countries should share H5N1 and other flu strains that have pandemic potential with the collaborating centers in a "rapid, systematic and timely manner." That's "great" for flu research and public health, says Richard Webby, the director of the WHO collaborating center at St. Jude Children's Research Hospital in Memphis, Tennessee. "It's very important that we work with the most relevant isolates available," Webby says. "Otherwise, it's kind of useless."

On the flip side, companies that produce flu drugs, vaccines, and diagnostics will



Odd one out. Former health minister Siti Fadilah Supari refused to share samples of H5N1, which hit Indonesia hard, in 2006.

shoulder 50% of the costs of WHO's global influenza surveillance network; the total tab was about \$57 million in 2010. (A special advisory group will determine how much each of them pays.) If there's a pandemic, the companies must also pick two items from a menu of obligations that includes things like donating at least 10% of their vaccine production to WHO, or granting royalty-free licenses on their intellectual property (IP) rights to manufacturers in developing countries. Some companies voluntarily made such donations during the H1N1 pandemic, but "this introduces a level of predictability," Fukuda says.

The agreement also addresses complaints from scientists in developing countries that they get few professional rewards for collecting and sharing samples that sometimes help land papers in prestigious journals. Recipients of virus isolates must "actively seek" the participation of scientists in developing nations in research and engage them when writing and publishing the results.

The agreement is a significant step, says Sangeeta Shashikant of the Third World Network, an advocacy group; until now, countries couldn't expect anything in return for their compliance with WHO's scheme. But

the 10% donations are too little, she says, and the royalty-free IP licenses should have been compulsory, not just one of the choices on the menu. Fidler says protecting IP was the pharmaceutical industry's main goal in the negotiations. "Once that flank was safe, they felt comfortable," he says. In a statement last week, the International Federation of Pharmaceutical Manufacturers and Associations "welcomed" the agreement.

Fidler finds it "very curious" that the agreement makes few demands on rich countries' governments. They could have been obligated or encouraged to donate 10% of any pandemic vaccine supplies to the developing world, as some countries did during the H1N1 pandemic in 2009–10. But Fukuda says they are already making other contributions, such as funding labs and regulatory agencies and helping to establish vaccine factories in the developing world.

To David Fedson, a retired pharma executive and a campaigner for pandemic preparedness, the deal doesn't address the most important issue: the huge vaccine shortage that inevitably arises during pandemics. WHO could urge companies to produce only so-called adjuvanted vaccines, which need far less antigen per dose, he says, or accelerate development of a new generation of vaccines that are easier to produce.

How the agreement will work in practice remains to be seen. The text contains a lot of nonbinding language, Fidler says, such as "should," "are urged," or "will encourage." "As an international lawyer, you're looking for 'shall,' 'will,' and 'must,'" Fidler says. In theory, Indonesia could still refuse to share samples because all the text says is that it "should" do so, Fidler says. Fukuda says this kind of language is common in global health cooperation, but that doesn't mean the agreement has no teeth. "We won't send an army" to countries that ignore the agreement, "but after 4 years of negotiations, this carries a lot of weight."

As to Indonesia, its refusal to share only applied to H5N1; during the H1N1 pandemic, it has dutifully sent its virus to the WHO labs. And although many countries have given moral support to Indonesia's stance on H5N1, none has followed its example. Once the new agreement takes effect, Fukuda expects Indonesia to fully return to the international fold.

—MARTIN ENSERINK



Are Dolphins Too Smart for Captivity?

A new movement seeks to end all dolphin research in zoos and aquariums, but critics say that could kill a productive field and hurt these animals in the wild

TAB AND PRESLEY WERE YOUR TYPICAL show dolphins. They spent their days at Brooklyn's New York Aquarium gliding through the turquoise water of their large outdoor pool, rocketing into the air for cheering spectators, and being ogled through thick windows of Plexiglas. But in 1998, they had the chance to participate in a revolutionary science experiment.

Working in the morning before shows, cognitive psychologist Diana Reiss scribbled a few black triangles and circles on Tab's and Presley's foreheads, backs, and flippers—all places the bottlenose dolphins couldn't see. Then, while biopsychologist Lori Marino watched from afar, Reiss put a mirror in the

tank. The two dolphins swam to it and immediately began checking out their new tattoos, twisting their bodies so they could make out every mark.

Though it seemed a simple behavior, Tab and Presley had just done something extraordinary. They had shown that they could recognize their own reflections—a test of self-awareness that only chimpanzees and humans had passed at the time.

The finding was a breakthrough in dolphin research and a milestone in the field of animal cognition. But it also sowed an uncomfortable seed in Marino's mind. If dolphins were as self-aware as people, she recalls thinking, how can we keep them locked up in concrete pens?

Marino tried to repress the thought. The science, she told herself, had to come first. But when she and Reiss attempted to continue the mirror studies a couple of years later, they learned that Tab and Presley had been transferred to other aquariums where both had died of infections at about 20 years of age, half a dolphin's normal life span in the wild.

The deaths affected Marino profoundly. In the following years, she abandoned her aquarium work, severed her relationship with Reiss, and launched a crusade to free all dolphins from captivity.

Marino is swimming upstream, however. Although her colleagues are concerned about the welfare of dolphins, most have concluded that captive research is both the best way to learn about the intelligence of these creatures and the best way to protect them in the wild. "There are always ethical issues with working with animals in captivity," says Richard Connor, an animal behaviorist at the University of Massachusetts, Dartmouth, who studies wild dolphins in Shark Bay, Australia. "But what Marino is proposing would completely kill the field of dolphin cognition."

Early show. Some of the first work on dolphin cognition took place at Florida's Marine Studios, now Marineland.

Flipper's brain

Tab and Presley may have proved that dolphins have brains to be reckoned with, but their predecessors were a black box to early researchers. The first serious studies of dolphin intelligence took place at a decidedly nonserious location: Marine Studios, an aquarium on Florida's northeast coast that served as the backdrop for B movies such as 1954's *Creature from the Black Lagoon*. (Its name was later changed to Marineland.)

During a visit to the park in 1955, a pioneering brain researcher named John Lilly became fascinated with its bottlenoses and set up a research lab there. His early experiments were crude. He hammered electrodes into the skulls of live dolphins, for example. But he also performed less invasive studies, analyzing the animals' chirps, whistles, and other sounds. Lilly's work convinced him that dolphins were highly intelligent and possessed a complex vocabulary—findings that enticed a new breed of researcher to study them.

By the 1960s, the U.S. Navy had invested heavily in dolphin research. Its primary interest was militaristic, training some of its first dolphins to recover practice rockets and mines from the sea floor. Working in large, open-water pens, the Navy also made major advances in understanding dolphin physiology and echolocation. (Using a focused beam of clicks, dolphins can find a fish buried in the sand from several meters away.)

Meanwhile, Lilly had begun to wander off the deep end. Although his work had captured the public's imagination—inspiring movies like 1963's *Flipper* and helping foster the rise of marine parks—he began making increasingly fantastic claims, arguing, for example, that dolphins held the key to communicating with extraterrestrials. He also gave some dolphins LSD in an attempt to talk to them. Lilly's behavior threatened to torpedo the entire field.

But dolphin research found a savior in Lou Herman, a former Air Force intelligence officer who founded a research-only dolphin facility in Honolulu, Hawaii, in 1970. Working in former shark tanks, Herman showed that dolphins could understand two artificial languages—one based on electronic sounds, and the other on a trainer's hand gestures—and that they grasped grammar and syntax. They could even create their own novel behaviors on command and comprehend human pointing, a skill that eludes chimpanzees.

By the time Herman closed his lab in 2004, he had seeded the field with countless researchers, many of whom continue to demonstrate additional signs of dolphin intelligence, including tool use and cultural traditions. When Marino had her epiphany at the New York Aquarium, it wasn't just the deaths of Tab and Presley that moved her. It



Deep thoughts. Lori Marino's work on dolphin brains helped convince her that the animals were too sentient for captivity.

was the mounting weight of decades of this research suggesting that the dolphin brain isn't that much different from our own.

The crusade against captivity

In late January of 2006, a young bottlenose dolphin named Harley jumped out of his tank at the Minnesota Zoo and smacked his head on the surrounding concrete. His trainers, assuming nothing was wrong, returned him to the water, where he soon stopped coming up for air. By the time divers reached him, Harley had died, the victim of a fractured skull.

Marino, now at Emory University in Atlanta, says such incidents illustrate the ills of captivity. Dolphin tanks are chemically treated, bereft of other marine life, and just a tiny fraction of the hundred-square-kilometer ranges these animals are used to, she says. In these bland and sterile environments,

Marino says, it's no wonder that intelligent, social dolphins swim in circles and jump out of their pools, and that these stresses contribute to their premature deaths from gastroenteritis, fungal infections, and other ailments: "You can't replicate the natural settings for these animals."

A 2009 report sponsored by The Humane Society of the United States and the World Society for the Protection of Animals came to a similar conclusion. Citing figures from marine parks and aquariums, the U.S. National Marine Fisheries Service, and longitudinal studies of wild dolphins, *The Case Against Marine Mammals in Captivity* found that between 5.6% and 7.4% of dolphins die each year in captivity versus 3.9% in the wild. The numbers for orca whales are even more dramatic: 6.2% to 7% mortality rates in captivity versus 2.3% in the wild. "The totality of the captive experience for marine mammals is so contrary to their natural experience that it should be rejected outright," the report concludes.

After Tab and Presley died, Marino began refusing offers to work in aquariums and studied only dolphins that had already died. Conducting MRI and CT scans on stranded wild dolphins, she found that—adjusted for body size—the animals sported the second biggest brains on the planet, larger than chimps' and just below humans'. She also discovered that dolphins have a very complex neocortex, which has been linked to problem solving, self-awareness, and processing emotions in people (*Science*, 26 February 2010, p. 1070).

Armed with these data, "I couldn't in good conscience continue to support captive research," Marino says. She now calls herself a "scholar advocate" and has gathered allies, such as Denise Herzing, a psychologist at Florida Atlantic University in Boca Raton, who works only in the wild. Other dolphin researchers have objected to captivity, Herzing says, but like her they simply moved their studies outdoors. What's different about Marino is that she's trying to foment a revolution.

Marino has also teamed with advocacy groups like TerraMar Research, a nonprofit organization based in Seattle, Washington, dedicated to protecting marine wildlife. TerraMar's director, Toni Frohoff, argues that if dolphins are as self-aware as people, they deserve the same basic rights. "The more sentient we see dolphins to be," she says, "the greater our ethical obligation to them. We can't study them like goldfish or lab rats."

Taking a cue from the Great Ape Proj-

Online sciencemag.org

 Podcast interview with author David Grimm and an online discussion on ScienceLIVE.

ect, a collection of scientists and advocates who have argued that chimps and their relatives deserve basic legal rights (*Science*, 1 April, p. 28), Marino banded together with other scientists, activists, and philosophers to draft a “Declaration of Rights for Cetaceans” in 2010. It states that no cetaceans—a group that includes whales and dolphins—“should be held in captivity ... or removed from their natural environment.” Instead, live cetaceans should only be studied in the wild. Marino and her allies have gathered more than 3200 signatures and hope eventually to bring the declaration before the United Nations. “We want to use this as a jumping-off point for changing policy,” Marino says. “We need to move the science to a place that doesn’t compromise our ethics.”

Counterattack

Stan Kuczaj hadn’t even heard of Marino’s fledgling movement when, in 2010, he commissioned a barrage of articles on marine animals for the *International Journal of Comparative Psychology*, which he edits. His target was the 2009 Humane Society report, and over the course of two issues and 600 pages, some of the biggest names in the field argued that captive research was critical to understanding dolphins and other marine mammals and to protecting them in the wild.

Kuczaj, a psychologist at the University of Southern Mississippi in Hattiesburg who studies dolphin behavior and communication in captivity and the wild, also feels that the papers are an effective rebuttal to Marino and her allies. “I don’t think there are unequivocal data to support some of the claims that they make,” he says. He agrees that dolphins are smart, for example, but says there’s no evidence that they’re comparable to people. “We suck at being able to validly measure intelligence in humans. We’re even worse when we try to compare species,” he says. Marino and her allies are “trying to push their personal opinion on the field.”

Herman agrees. The godfather of research on dolphin cognition and a contributor to the journal package, he says that the evidence for higher dolphin mortality in captivity versus in the wild is “very, very questionable,” adding that a recent study based on National Marine Fisheries Service data showed no significant difference. “The mortality is horrific in the wild. Fifty percent of wild dolphins bear shark scars—and those are the ones that are still alive.” Marino, he says, reminds him of John Lilly,



Finned soldier. Navy research has revealed much about the physiology of dolphins.

who eventually railed against captivity as a concentration camp: “Once you mix politics with science, you lose objectivity.”

Herman says he, too, has struggled with the ethics of keeping dolphins in captivity. But he notes that Marino is basing many of her ethical arguments on understanding gained from captive research. “That’s the irony of it. How do they know dolphins are intelligent? Because of the captive studies. And now they don’t want us to do that research.” Herman says he never could have made his cognitive breakthroughs in the wild. Researchers have to train animals, collect baseline readings, and follow individuals for months or years, he notes: “Science demands controls and replication. What they’re proposing is a fantasy.”



Getting the message. Lou Herman’s work in a Hawaii facility has shown that dolphins grasp grammar and syntax.

And are dolphins really unhappy in captivity? Dorian Houser, director of biological research at the National Marine Mammal Foundation in San Diego, California, doesn’t think so. “Humans have forgotten what it’s like to forage for their own food and be the prey item of another animal,” says Houser, who has worked with Navy dolphins. “These animals get three square meals a day and regular medical care that’s probably better than most people have.” In San Diego Bay, the only thing that separates the Navy’s open-water pens from the rest of the ocean is a small walkway, he says, which the dolphins could easily jump over—but don’t.

Plus, says Houser, information gleaned from captive studies has helped scientists understand dolphins’ sensitivity to noise, pollution, and other dangers—all of which have helped the Navy draft better conservation guidelines—while decades of research on dolphin physiology has helped rescuers save stranded animals. Captive research, he notes, is also heavily regulated by a variety of government agencies, a position echoed by Marilee Menard, the executive director of the Alliance of Marine Mammal Parks and Aquariums, which represents 55 facilities around the globe. “These animals are stress-free, they’re reproducing fabulously,” she says. “What other standards do you want?”

The biggest concern among those who study dolphins in captivity, however, is that removing the animals from zoos and aquariums would destroy the field of dolphin cognition. In the past decade, researchers have made astounding cognitive discoveries in other animals, showing, for example, that jays can plan ahead and dogs understand



Marine lab. Denise Herzing studies dolphins in the Bahamas, where the animals come and go as they choose.

inequity. “Well, dammit, we need to have that looked at in dolphins,” says Connor of the University of Massachusetts. “We’ve just scratched the surface of figuring out what these animals are capable of,” he says. “You can’t just say, ‘Hey, a dolphin recognizes itself in the mirror,’ and go home.”

Still, Connor says he is open to exploring ways of studying cognition in the wild: “If they can do these studies in free-ranging dolphins, great. But the burden of proof is on them.”

A new paradigm

Marino and her crew plan to spend the next several years overcoming that burden. But even in the wild, they want ethics to be paramount. That means working only with whales and dolphins that have “decided” to interact with humans—either because they’re curious or because they’ve become used to people—a controversial approach Marino and Frohoff call “collaborative research.”

Their model is the work of Florida Atlantic University’s Herzing. For more than 2 decades, Herzing and her colleagues have been hanging out on a catamaran in the Bahamas, about 65 kilometers from shore. The water is shallow and clear, and two communities of dolphins come and go as they please. The researchers have been there so long that the dolphins ignore them, as habituated great apes and elephants ignore human observers in the wild. Snorkeling

nearby, Herzing and her colleagues use cameras and hydrophones to record behaviors and sounds, and collect genetic data from feces, all without interfering with the animals. In 2008, her team showed that mothers teach calves how to fish, considered the highest form of social learning and usually thought restricted to primates. “We want to get scientists thinking about new tools and new techniques,” Herzing says. Kuczaj says she has been “living the goal” of doing good cognitive work in the wild.

Marino herself is working on adapting several captive-research protocols to the wild. She’d like to see, for example, if she can replicate her mirror studies in the open ocean by modifying the techniques she and Reiss used at the New York Aquarium.

While Marino and her allies work out the details, they’re also developing an ambitious plan to phase out all whale and dolphin captivity in the United States. They hope their efforts will sway public sentiment against the 30 U.S. marine parks and aquariums, forcing them to close their dolphin and whale exhibits, which house about 400 animals. Herzing dreams of creating some sort of “retirement center,” such as a lagoon where these animals could live with other members of their species until they died, similar to chimp and elephant sanctuaries.

Marino says her ultimate goal is to convince the next generation of dolphin researchers that they don’t need to work in

captivity. She wants them to go straight to the wild, without having to endure a Tab and Presley moment first.

Reiss, who continues to work with dolphins in captivity—a position that drove Marino to stop speaking to her in 2009—doesn’t support Marino’s movement, arguing that there’s still value in captive research. But Reiss does think dolphin researchers can find common ground. Now at Hunter College in New York City, she is spearheading a campaign to stop the bloody dolphin hunts in Taiji, Japan, for example. Marino joined this effort, as did scientists on both sides of the captivity debate. Reiss and others who support captive research also believe that many zoos and aquariums should improve their dolphin facilities. Everyone wants the best for these animals, Reiss says: “To me, the biggest thing is to keep the knowledge coming, whether they’re in captivity or in the wild.”

The issue reaches far beyond dolphins, says Karen McComb, a behavioral ecologist at the University of Sussex in the U.K. who has chosen to study elephant cognition in the wild rather than in zoos. The more we learn about the intelligence of other animals, she says, the more we’re forced to consider the ethical implications of studying them. “Everyone is coming to a point where it’s relevant to stand back and reconsider these issues,” she says. “It’s a really important debate.”

—DAVID GRIMM



Expert and witness. Phil Zimbardo talks about the psychology of evil at Stanford University in 2007.

SOCIAL PSYCHOLOGY

Using the Psychology of Evil To Do Good

Forty years after the infamous Stanford Prison Experiment, Phil Zimbardo thinks he can apply its lessons to teach ordinary people to be heroes

When the disturbing photos of American soldiers mistreating prisoners at Abu Ghraib prison in Iraq began leaking out 7 years ago this week, they jolted Philip Zimbardo with a sickening sense of déjà vu. The nakedness of the prisoners and the bags over their heads, the shamelessness and perverse creativity of the captors—he'd seen much of this before. In 1971, as a psychology professor at Stanford University in Palo Alto, California, Zimbardo presided over one of the best-known experiments in the history of social psychology. He sequestered 24 student volunteers in a boarded-up corridor in the basement of Jordan Hall, randomly assigning half to be guards and half to be prisoners. Six days into an experiment that was supposed to run for 2 weeks, Zimbardo had to call it off because the prisoner abuse had gotten so bad.

By 2004, Zimbardo had put the Stanford Prison Experiment behind him. But as the Abu Ghraib story unfolded, he slowly became a part of it, first through media interviews on prison psychology, and then as an expert witness in the trial of staff sergeant

Ivan "Chip" Frederick, the highest ranking soldier charged for the abuse. The experience caused him to reexamine the prison experiment and question his own role in it.

It also gave Zimbardo inspiration for his next big experiment. At age 78, he has reinvented himself as a social entrepreneur, leading a new project that will attempt to turn the Stanford Prison Experiment and other studies of the dark side of the human psyche into a force for good. Last year, Zimbardo founded the Heroic Imagination Project, which operates out of a former Army post in San Francisco's Presidio park. Its goal is to use lessons learned from social psychology research to teach ordinary people—from high school students to office workers—to recognize the influences that can make people stand idly by when they see a person in need and embolden them to commit what Zimbardo calls acts of everyday heroism. The organization has plans for teaching, research, and public outreach that are just getting off the ground. "Our ambition is to seed the world with heroes," Zimbardo says.

Taking prisoners

To mark the 40th anniversary of the Stanford Prison Experiment this summer, the American Psychological Association will host a symposium on the legacy of the experiment at its annual meeting. The original goal was to investigate what happens when good people find themselves in a bad situation. "We went to great lengths to put good apples in a bad barrel," Zimbardo says. All the guards (and prisoners) were healthy young men with no history of psychological problems, drug abuse, or run-ins with the law. The question was: "Does the goodness of the apples dominate, or does the badness of the barrel corrupt?"

The first day passed without incident. But on the second day the prisoners rebelled, taking off their numbers and barricading themselves in a cell. The guards called in reinforcements, conducted strip searches, and put the ringleaders in solitary confinement. The guards' treatment grew harsher in the following days as they forced prisoners to do pushups, limited their access to the toilet, and used psychological tactics to break down solidarity. Thirty-six hours into the experiment, one prisoner suffered a breakdown, crying, screaming, and cursing. Zimbardo suspected he was faking but sent him home.

Over the following days, Zimbardo says at least 50 people came to see what was going on in the basement of Jordan Hall, including colleagues in the psychology department and parents and friends of the prisoners. Most didn't see the worst of the abuses, but

CREDIT: PAUL SAKUMA/AP PHOTO

few raised questions about the experiment and none suggested it be stopped. A Catholic priest who had been a prison chaplain met with the prisoners; afterward he congratulated Zimbardo on the realistic prison environment he'd created. The lines between role-playing and reality were becoming blurred to say the least. When Zimbardo looks back at video of the experiment, he sees himself pacing the corridor with his arms stiff behind his back, like a general reviewing his troops. "I started out being the principal investigator of the Stanford Prison Experiment, and over time I became the superintendent of the Stanford Prison," he says.

He didn't realize the change that had overcome him until Friday night, almost halfway through the scheduled 2 weeks. He'd invited his new girlfriend, Christina Maslach, who'd just finished her Ph.D. at Stanford, to come see the experiment and go out to a late dinner. Maslach arrived around 10 p.m. as the guards were leading the chained and hooded prisoners on their final toilet break of the day. She was stunned to see one of the guards, with whom she'd chatted amiably on her way in, bullying the prisoners. She ran out in tears.

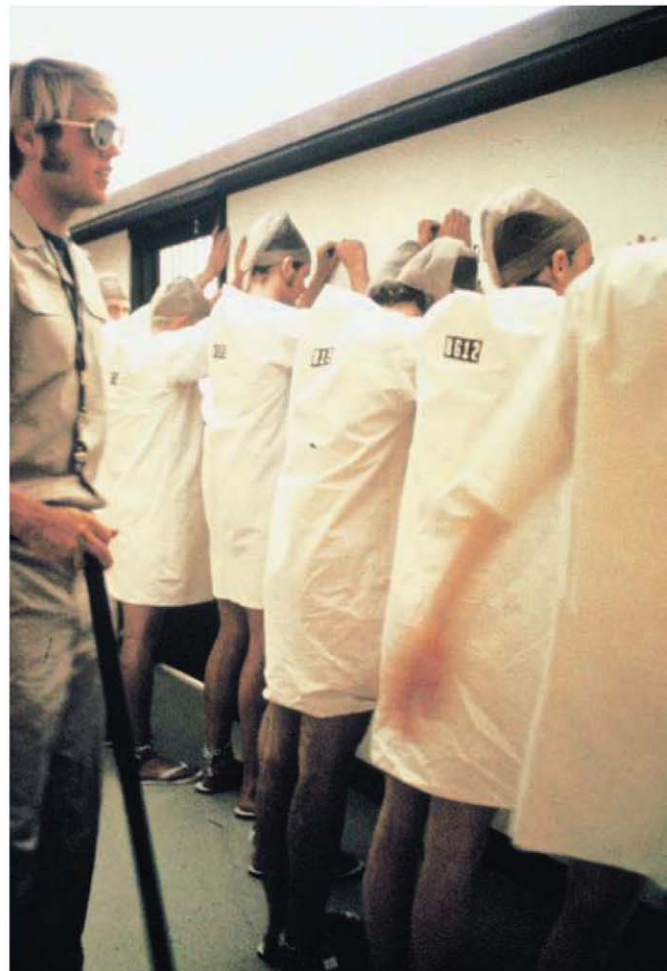
When Zimbardo caught up with her outside the building, he chided her for getting so emotional. She was incredulous. "How can you not see what I see?" he remembers her asking. "Those are not prisoners, those are not guards—those are boys who are suffering and you're responsible," Maslach told Zimbardo. "She said, 'I'm not sure I can continue dating you,'" Zimbardo says. After a long argument, he realized she was right, and he ended the experiment the next morning. Zimbardo and Maslach got married a year later, and they're still married today.

An unwelcome flashback

By 2004, Zimbardo's research interests had turned to shyness, time perception, and other topics. He'd written a widely used introductory psychology textbook and narrated an award-winning public television series on psychology. "It wasn't until Abu Ghraib that the whole thing came up again."

As he watched the news, Zimbardo was appalled to hear military leaders insist that the abuse was the work of a few bad apples. "They couldn't have known that," he says. For one, right after the first incidents came to light there hadn't been time to determine how widespread the abuse had been. For another, Zimbardo knew that even good apples can be corrupted by a bad barrel. And Abu Ghraib was a bad barrel if there ever was one, he says.

Zimbardo tried to explain this, via video link, to a military court in Baghdad as an



Tough tactics. Guards in the Stanford Prison Experiment used blindfolds, forced nakedness, and roll calls to assert their authority and control over the prisoners.

expert witness for the defense in Frederick's trial in the fall of 2004. An Army reservist with a previously laudable military record, including more than a dozen medals and other awards, Frederick shipped out for Iraq in October 2003 and soon found himself supervising about a dozen military police and dozens more Iraqi police responsible for guarding more than 1000 Iraqi prisoners at Abu Ghraib. The prisoner population had recently tripled, creating a chaotic environment in which standard procedures and oversight broke down. The language barrier made prisoners seem anonymous, Zimbardo says, and many prisoners were forced to go naked, further dehumanizing them and creating a sexually charged atmosphere. Guards worked daily 12-hour shifts for weeks on end. Fear of a revolt—or an attack from outside—mixed with boredom and exhaustion to create a volatile brew.

In a deal with prosecutors, Frederick pleaded guilty to five charges of abuse and dereliction of duty. Zimbardo testified that the situation Frederick was placed in, with-

out remotely adequate training, was a powerful influence on his behavior and should be considered a mitigating factor. He doesn't think the message got through. The Army stripped Frederick of his medals and pension, gave him a dishonorable discharge, and sentenced him to 8 years in prison. The heavy sentence and the fact that Frederick, a relatively lowly staff sergeant, was the highest ranking officer charged in the entire scandal still rankles Zimbardo. "He clearly got scapegoated," he says.

Psychology for the people

In his 2007 book, *The Lucifer Effect*, Zimbardo recounts his investigation into Frederick's case and his reexamination of the Stanford Prison Experiment. Reviewing the tapes of the experiment, Zimbardo saw how his own passive role enabled the abuse. That pained him. But it also forced him to contemplate how he might use his knowledge and expertise to do something good.

The Heroic Imagination Project (HIP) is the result. The grandiose name is matched by



Applied psychology. Civics teacher Devin Carberry talks about the bystander effect in relation to the Holocaust with his high school class in Oakland, California.

an ambitious slate of projects in various stages of development. The basic idea behind all of them is that anyone can be a hero. In Zimbardo's view, people too often think of heroes as celebrities or exceptional people in exceptional circumstances, like the firefighters who rushed into the burning towers on 9/11 or the pilot who safely landed his hobbled plane in the Hudson River. He insists on a different definition: any ordinary person who takes action to help somebody in need (or in support of a moral cause), at some risk to himself and with no expectation of personal gain. One of his favorite examples is Daniel Ellsberg, the former military analyst who in 1971 leaked the top-secret Pentagon Papers to the press, making public for the first time damaging revelations about U.S. government conduct during the Vietnam War.

Taking this message to young people is a major aim of the project. One semester-long pilot program is just coming to an end at ARISE High School in Oakland, California. There, HIP's director of education, former high school principal Clint Wilkins, is working with ARISE civics teacher Devin Carberry to weave some social psychology into his class on the Holocaust. The goal is to help students understand what happened in Nazi Germany and recognize the influences, for better or worse, they face in their own lives.

Students have watched clips on classic findings in social psychology. In one, Zimbardo explains Stanley Milgram's infamous experiment on obedience to authority. In 1962, Milgram (coincidentally, a former high school classmate of Zimbardo's) told volunteers at Yale University they were participating in a study on learning. The volunteers were assigned to be the "teacher" and

instructed by a researcher in a lab coat to flip various switches to shock the "learner"—visible through a window in an adjacent room—to punish incorrect answers on a memory test. They did not know that the learner was a confederate and not hooked up to live wires. As the experiment progressed, nearly two-thirds of the volunteers followed orders and delivered the maximum shock, despite screams of apparent pain from the learner. The result is a chilling demonstration of the power of a situation to corrupt ordinary people, and it lent support to Milgram's fear that the Holocaust could happen again. The first step to becoming a hero, Zimbardo says, is recognizing the social pressures at work in a given situation.

On a recent afternoon, Wilkins stood in front Carberry's class, reminding the students of another video they'd watched about Kitty Genovese, a 28-year-old New York City woman stabbed to death near her home while her neighbors looked on. Genovese's murder, in 1964, spurred dozens of psychological studies on what became known as the bystander effect, when people who see someone in need of help are less likely to intervene if others are nearby, partly because they assume someone else will. "We have a fancy name for that, we call it the diffusion of responsibility," Wilkins says.

Carberry wants the students to relate this to the Holocaust. They've just read *Night*, Elie Wiesel's book based on his family's experience in the concentration camps, and a girl with low bangs and hoop earrings mentions a scene in which a child is hanged in front of the prisoners. "That's right," Carberry says, "there was a child there on the block, and how many onlookers were there?"

A collective murmur goes up from the class. "Hella." (California-speak for "a lot.") They debate whether it would have done any good for any one of them to intervene.

Wilkins asks the class whether knowing about the bystander effect would make them more likely to act if they saw an attack. It's not an outlandish scenario. Most of the students live in inner-city neighborhoods that see more than their share of violence. The morning after Wilkins's visit, the owner of a popular Mexican restaurant a few blocks from the school was shot and killed as he opened his restaurant. "If there were a knife fight in the plaza out here in front of the school, would you be more likely to get involved?" Wilkins asks. "Hell no!" someone blurts out. "Okay, that's probably smart," Wilkins says, "but what could you do without putting yourself in danger?" The discussion continues. It's not clear what lessons the students will take with them, but Carberry says the added dose of psychology seems to have kept them more engaged. "The kids really seem to dig it," he says.

Back at the Presidio, Zimbardo enthusiastically reels off a seemingly endless list of projects in the works. HIP has just finished its first study, a collaboration with researchers at Vrije Universiteit Amsterdam in the Netherlands on the psychology of whistle-blowing. In a contrived setup in which volunteers had a chance to notify the university's committee on human subjects of an unethical experiment, only 6% blew the whistle. Now the team is investigating what personality traits and motivations distinguish the whistleblowers. "There's almost no research on any of these questions," Zimbardo says. HIP is also developing corporate training programs and a host of Web sites to encourage people to post stories about their own personal heroes and take on "hero projects" like getting a friend to quit smoking or helping an elderly person learn to use a computer.

Zimbardo brushes off the suggestion that it all seems a bit scattershot. "This is my new mission in life," he says. He chipped in \$30,000 of his own money to start the project and has since raised nearly \$250,000 more from other donors. He's considering auctioning off some of his art and wine collections. "I grew up in abject poverty in the South Bronx," Zimbardo says. Now that he has nice things, he says he's willing to give them up if that's what it takes. Zimbardo seems to have thrown himself wholeheartedly into the challenges of his grand new experiment—and the shot at redemption. "It's rescued my career from being Dr. Evil to being Dr. Good," he says.

—GREG MILLER

CREDIT: G. MILLER/SCIENCE

NEWSMAKER INTERVIEW

Jeremy Berg: An Independent Scientist Departs NIH's Ranks

The outgoing director of NIH's basic research institute has won scientists' hearts and minds by championing transparency

Jeremy Berg has a knack for making his life complicated. Soon after he took the helm of the U.S. National Institutes of Health's (NIH's) basic science institute in 2003, Berg questioned why the winners of a prestigious new award for creative research were all well-known male scientists. So NIH asked him to run the program. When scientists devoured data he shared on how his institute awards grants, he found himself launching NIH's first director-written blog. Last December, as a member of a high-level advisory board, he cast the only vote against creating a new NIH institute to bolster drug development. Berg's concern: Forming the new institute would likely mean dissolving another NIH center that's treasured by biomedical researchers. It provides resources like large instruments and animal models.

Berg rushed from the room after the 12-1 vote, visibly upset. "I was really fairly stunned that there would be so little discussion," says the director of NIH's \$2 billion National Institute of General Medical Sciences (NIGMS). But "as an army of one, I wasn't going to get very far. I was just trying to digest the whole thing."

Berg's ongoing battle to defend NIH's National Center for Research Resources (NCRR) has burnished his image among many scientists who were already fans. "I admire his courage. I think he was absolutely correct," says cell biologist Keith Yamamoto of the University of California, San Francisco, who calls NIH Director Francis Collins's decision to abolish NCRR without input from his advisers "pretty distressing."

In June, Berg, age 53, will leave NIGMS for the University of Pittsburgh to follow his wife, breast imaging researcher Wendie Berg. He will run a biochemistry lab half-time and serve as "associate senior vice chancellor for science strategy and planning in the health sciences." (Details are still being worked out, he says.) Last month, in his small office crammed with family photos and dangling metal models of proteins, Berg reflected on his time at NIH.

After a junior faculty position at Johns Hopkins University, Berg became a biophysics department chair at just 32. He "had done

some beautiful work" on the structure of zinc finger proteins and had "leadership written all over him," says then-cell biology chair Thomas Pollard, now dean of arts and sciences at Yale University. Berg, who served in several administrative posts at Johns Hopkins, says he enjoyed making organizations more effective and "reveling in other people's success." When Johns Hopkins scientist Elias Zerhouni became director of NIH, he recruited Berg to head NIGMS.



Dissenter. Berg questioned the NIH director's abrupt decision to abolish an institute.

The institute was in good shape—its budget had nearly doubled in 5 years—but Berg saw a need to clear up "confusion" about how NIGMS decides which peer-reviewed grant proposals to fund. Scientific quality scores aren't the only factor; the institute also considers how a proposal fits its programmatic goals. Berg began e-mailing NIGMS grantees each year's funding curve—bar graphs showing that while the best-scoring grants are usually funded, some lower-rated ones are, too. "It's information people have a right to know," Berg says. The newsletter became a popular blog; it inspired NIH extramural research chief Sally Rockey to launch her own blog this year on funding policies.

Last fall, Berg examined how lab size relates to productivity. He found that the average scientist's output of papers peaks at roughly \$700,000 a year in costs, or about three research grants. Although NIGMS already had a policy of spreading the wealth and not automatically awarding grants to already well-funded scientists, the analysis gave this policy "a strong factual foundation," Berg says. Stanford University's Suzanne Pfeffer, who as president of the American Society for Biochemistry and Molecular Biology bestowed an award on Berg last year, praises him for "making sure that the little guys get paid."

Berg was drawn into broader NIH matters after he pointed out that the winners of the first round of Pioneer Awards, a new grant program for high-risk research, were mostly "the usual suspects," Berg says. He made small changes to the nomination process and the roster of reviewers that resulted in "a better balance of age groups and gender," Berg says. He helped lead other NIH-wide tasks, such as an overhaul of peer review. "Jeremy has been an extraordinarily good citizen, willing to take on complicated and politically sensitive issues. And he's done a spectacular job," says Story Landis, director of the National Institute of Neurological Disorders and Stroke.

Berg's role switched from model citizen to dissident, however, when Collins and other NIH leaders decided to abolish NCRR. They reasoned that because NCRR's large clinical program was moving to the new National Center for Advancing Translational Sciences, the rest of NCRR should be parceled out to other institutes. What irks Berg is the process: Collins did not consult with the scientific community through his Scientific Management Review Board in advance. "This is the first time in the history of NIH that a center has been abolished, and to do it in this manner is a very bad precedent. ... If this is representative of a leadership style, it is going to impair the effectiveness of the organization," Berg says.

Berg wants to finish a few things before he leaves, including a review of NIGMS's training programs. It could require that institutions certify that they're paying attention to young scientists' career plans (*Science*, 4 February, p. 525). He's also taking a look at how well peer-review scores predict performance, comparing them with an investigator's productivity. It's a task he clearly enjoys. "I'm fundamentally a data person," he says. The results "are rich and complicated," says Berg, who plans to submit them to a journal.

—JOCELYN KAISER

MEETING BRIEFS >>

PALEOANTHROPOLOGY SOCIETY & AMERICAN ASSOC. OF PHYSICAL ANTHROPOLOGISTS | 12–16 APRIL 2011 | MINNEAPOLIS, MN



Hall of fame. Lee Berger showed off casts of *Au. sediba*'s bones in the hallway at the meeting.

A New Ancestor for *Homo*?

Finding one partial skeleton of an ancient member of the human family is the rarest of rare discoveries in human evolution. So paleoanthropologists in the audience murmured in surprise when South African researchers announced at a talk that they had found bones and teeth from at least four individuals of a new species of early human, *Australopithecus sediba*. The discoverers say this species shows some surprisingly modern traits and may even be an ancestor of our own genus. “We really have found something very, very odd and very unexpected,” says discovery team leader Lee Berger of the University of the Witwatersrand, Johannesburg, in South Africa. But other paleoanthropologists are waiting for more detailed analyses of the unpublished fossils before they agree on its identity or place in the human family tree.

The four hominin individuals died when they fell into a “death trap” in a cave at Malapa, South Africa, 1.977 million years ago, according to new dates reported by Darryl de Ruiter of Texas A&M University in College Station in his talk. In addition to the articulated partial skeletons of a youth and an older female unveiled last year (*Science*, 9 April 2010, p. 154), the team members also reported the discovery

of an 18-month-old infant and at least one other adult. This means they are getting a good look at *Au. sediba*'s development from infancy to old age.

In five separate talks, Berger and members of his team sketched a quick portrait of *Au. sediba*, who lived at the mysterious time right after the emergence of our genus, *Homo*, between 2 million and 3 million years ago. Researchers have long wondered which of several species of *Australopithecus* gave rise to *Homo*, with Lucy's species, *Au. afarensis*, as the leading candidate.



New face. The skull of *Au. sediba* shows some surprisingly modern features.

The trove of well-preserved bones includes clavicles, shoulder blades, and ribs as well as a largely complete skull, hand, foot, and two pelvises. The team calls the hominin an australopithecine because it had a small brain and “overall body plan” like that of an australopithecine, team member de Ruiter said in his talk.

But the fossils also show some surprisingly modern traits usually found only in members of our genus, Berger said. The two pelvises, in particular, are capacious and elongated, resembling those of *Homo*. In his talk, Berger ticked off a list of other modern traits, including smaller teeth, short hands, and an elongated thumb. In a separate talk

at the annual meeting of the Paleoanthropology Society, Kristian Carlson of Indiana University in Bloomington described the endocast—the impression left inside the skull by the brain—and suggested that the forebrain might be reorganized in a modern way. If so, *Au. sediba*'s brain and pelvis both would have begun to evolve into more modern shapes before the brain expanded, countering the view that a big brain drove the evolutionary remodeling of the pelvis and brain.

Members of our genus were already living when these hominins fell into the pit at Malapa, so these particular individuals aren't our ancestors. But de Ruiter said they might be late members of a species that previously gave rise to *Homo*, or a close relative.

Other researchers, who examined casts of the fossils at the meeting, agreed that on first glance they represent an unusual mix of primitive and more modern traits. But most thought it important to compare *Au. sediba* directly with other ancient hominins in more detail. “The pelvis does look more modern,” says paleoanthropologist Christopher Ruff of Johns Hopkins University in Baltimore, Maryland. “But that doesn't mean it looks exactly like a modern human's or that it gave rise to early *Homo*.”

Even if *Au. sediba* is an evolutionary dead end, says William Kimbel of Arizona State University, Tempe, “it does still shed light on the evolution of early *Homo*, because we know nothing about the time period a half-million to three-quarter million years before *Au. sediba*.”

Ancient Footprints Tell Tales of Travel

About 120,000 years ago, some three dozen men, women, and children stepped across wet volcanic ash on the ancient shores of Lake Natron in Tanzania. By scrutinizing their well-preserved trail, researchers have gotten their first snapshot of what a traveling group of archaic humans looked like, down to the size of the group and the ratio of men to women with children, according to paleoanthropologist Brian Richmond of George Washington University in Washington, D.C. “What's really exciting is we're getting a glimpse of actual behavior preserved in the

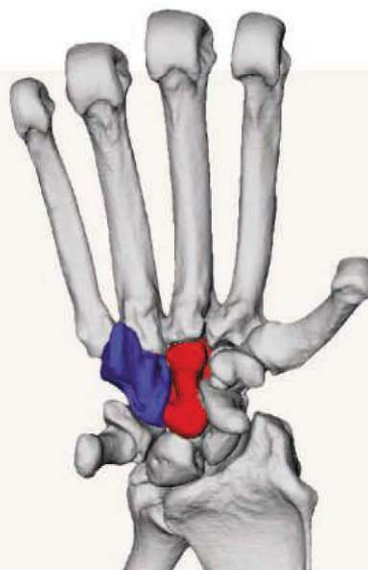
CREDITS (TOP TO BOTTOM): A. GIBBONS; PHOTO BY BRETT ELOFF; COURTESY OF LEE BERGER AND THE UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Snapshots From the Meeting >>

Finding *Homo*'s hips. What are the odds that fossil hunters would find a piece of a thighbone that fit perfectly on top of a broken bone found 29 years earlier? This is precisely what happened in 2009 at Koobi Fora on the east side of Lake Turkana in Kenya, where the famed Leakey family's teams have worked for decades: Researchers from the Koobi Fora Research Project fit the top of a newly found femur onto the shaft of the same individual's thighbone, which had been found back in 1980. In a talk, paleoanthropologist Carol Ward of the University of Missouri, Columbia, described the new thighbone and a pelvis found with it, which belonged to an early member of our genus that lived 1.88 million years ago.

The head of the femur is big and its neck is broad, and those features, plus others in the pelvis, reveal the fossil's owner as a member of *Homo*. But the shape of the femoral shaft shows it is not a member of *H. erectus*, a species known from Koobi Fora at this time. Finds of skulls and teeth had suggested that as many as three *Homo* species—*H. erectus*, *H. habilis*, and *H. rudolfensis*—coexisted at Koobi Fora from 1.8 million to 2 million years ago, but this is the first convincing evidence that those different *Homo* species had different postcranial morphology. "This gets us thinking about the diversity of creatures running around Koobi Fora nearly 2 million years ago," Ward says.

All in the wrist. Three new wrist bones from a second individual of the diminutive "Hobbit" from the Indonesian island of Flores confirm that the hand was too primitive to belong to a member of our species, *Homo sapiens*, according to a poster by paleoanthropologist Caley Orr of Stony Brook University in New York and his colleagues.



■ Hamate ■ Capitate

The other hand. These bones in the hobbit's wrist (drawn onto a chimpanzee hand) are different from those of modern humans.

They described three wrist bones found by Matthew Tocheri of the Smithsonian National Museum of Natural History among the unidentified bone remains of the hobbit, *H. floresiensis*; all the bones were discovered in Liang Bua cave on Flores. The wrist bones are smaller than those from the first *H. floresiensis* individual, but they show a nearly identical

pattern of unusual three-dimensional shape (*Science*, 21 September 2007, p. 1743). Both hands lacked features in a complex of bones, including the hamate and capitate, that mesh together to absorb stress in the hands of modern humans and Neandertals. This suggests that the hand bones of *H. floresiensis* did not distribute force away from the base of the thumb and across the wrist as efficiently as in modern humans.

Having the same features in two individuals' wrists makes it highly unlikely that their unusual shape was caused by disease in a modern human, as some researchers have claimed. "This is normal anatomy for *H. floresiensis*," Orr says. "It's consistent with the hypothesis that this is a distinct species."

—A.G.

fossil record," he said in his talk.

The local Maasai people have long known about the trail of footprints at Engare Sero on the southern shore of Lake Natron, in the shadow of Oldoinyo Lengai volcano, which is still active. But researchers didn't learn about the footprints—which are incredibly rare in the fossil record—until 2008. Since then, Richmond, geologist Cynthia Liutkus of Appalachian State University in Boone, North Carolina, and their colleagues have uncovered 350 tracks made by anatomically modern humans (as shown by their arched feet), over an area of 150 square meters. They noticed immediately that there are two sets of trails headed in opposite directions: one toward the northeast and another to the southwest. Preliminary radiometric dating on the volcanic ash below the prints puts them at about 120,000 years old. Because the footprints were made in wet ash, they must have been made in a matter of hours or days by "people living in a landscape at one place at one time," Richmond said in his talk.

Fancy footwork. Footprints in Tanzania reveal ancient travelers' behavior.



By scanning the footprints using photogrammetry, the researchers reconstructed a three-dimensional image of the site and used this image to measure precisely the length of each of the feet, which ranged from 14 to 29 centimeters. By counting how many different foot lengths were there, they estimated that more than 30 individuals laid down the tracks. Then they compared a couple of dozen of the foot lengths with those of more than 2000 African Americans to estimate the age and sex of each individual. They found, for example, that a large group of more than a dozen women and children with only one clearly identifiable adult male walked together from northeast to southwest. But the tracks that headed in the opposite direction (southwest to northeast) were made by several individuals who were not

traveling together, and there were more males. The tracks fit a scenario like this: A few men, women, and adolescents traveled in one direction. A short time later, a group of women and children and a man walked as a group in the opposite direction.

To test that idea, Richmond and his colleagues ran experiments examining the footprints left by local people who seldom wear shoes, as they walked and ran at various speeds. They concluded that people in the large group were all walking at the same speed, as if they all were adjusting their speed to travel together. But the people heading in the opposite direction were moving at different speeds, ranging from walking to running, suggesting that they were not moving together. The footprints provide "an extraordinary vignette of a moment in the lives of our ancestors," says paleoanthropologist Ian Tattersall of the American Museum of Natural History in New York City. And the prints freeze behavior. "Fossil bones have dominated interpretations of hominin locomotion," says paleoanthropologist Richard Potts of the Smithsonian National Museum of Natural History in Washington, D.C. "Now footprints are beginning to offer the real imprint of behavior."

—ANN GIBBONS

LETTERS

edited by Jennifer Sills

Toxicologists Welcome
Assessment Assistance

THE LETTER "ASSESSING CHEMICAL RISK: SOCIETIES OFFER EXPERTISE" by eight scientific societies (4 March, p. 1136) was the subject of lively discussion among the attendees at the annual meeting of the Society of Toxicology. Whereas some took this letter to indicate that its signatory societies regarded toxicologists as part of the problem concerning the limitations of the science involved in regulatory decision-making, a more generous view allowed that the signatory societies were expressing their interest in helping to assess the risks of chemicals and drugs by evaluating data and developing new testing guidelines and protocols.

As a scientifically integrated discipline, toxicology uses the tools of molecular and cellular biology, cancer biology, neuroscience, bioinformatics, reproductive and developmental biology, and other fields to determine how drugs and chemicals can be used safely. Toxicologists address the challenges of translating data from the molecular and cellular level into a better understanding of health

risks from contaminants in air, water, and food, and we urge all scientists to work collectively to meet that goal. In 2010, the Scientific Liaison Coalition (SLC), comprising 15 national scientific societies (including ours), was formed in order to increase the impact of the science of toxicology to improve public health. Toward this end, the SLC is already working on initiatives that address such issues as high-throughput testing in regulatory decision-making, the interactions between metabolic syndrome and environmental exposure risks, and the relationship between environmentally induced diseases and epigenetics.

Toxicologists and toxicology are already deeply involved in advancing regulatory science, improving toxicity testing, and protecting human health and the environment. We welcome the signatory societies of the 4 March Letter to join us in this effort.

SOCIETY OF TOXICOLOGY, ENVIRONMENTAL MUTAGEN SOCIETY,
TERATOLOGY SOCIETY

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Peaceful Collaborations
Deserve Support

THE FORMER RAND AFRIKAANS UNIVERSITY, now University of Johannesburg, has acted against its own and the South African national interest by terminating the 25-year-old collaboration with the Ben Gurion University of the Negev (Around the World item "End to partnership with Israeli university," News of the Week, 1 April, p. 19). Boycotts of Israeli scientists, science institutions, and worse,

research cooperation between Palestinians and Israelis [e.g., (1)] are counterproductive—they deprive those less fortunate of a chance for improvement in education, and they promote confrontation and tension between Arab and Israeli societies. Because of the political pressure they face, collaborative programs are dwindling. We should show strong support for the excellent examples of peaceful collaboration between Israeli and Palestinian scientists [e.g., (2–4)] as well as with scientists of other nations [e.g., (5, 6)].

WLADYSLAW ALTERMANN

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Prioritizing Taxonomists

ALARMING RATES OF SPECIES LOSS (1) COUPLED with a decline in the number of taxonomists (2) suggest that the estimates of species richness are far from complete. The problem lies less with the ability to collect specimens and more with the lack of resources available to identify them. Some recently published new species from India were collected 30 to 40 years ago (3). To expedite this process, we need to train more taxonomists by prioritizing taxonomy capacity-building programs (4).

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

CORRECTIONS AND CLARIFICATIONS

Reports: "The perception of rational, goal-directed action in nonhuman primates" by J. N. Wood *et al.* (7 September 2007, p. 1402). Wood *et al.* reported experiments on action perception with cotton-top tamarins, rhesus macaques, and chimpanzees. All of the research materials are available to support the findings from the tamarin and chimpanzee experiments. However, there are only summary data, as opposed to raw data, for the rhesus monkey experiments because the researcher who performed the experiments inadvertently failed to archive the original field notes. Upon realizing that the notes were unavailable, Wood and Hauser reran all of the rhesus experiments, using the same design and test population. Each trial was videotaped and coded blind to the experimental condition. They found the same pattern of results: Rhesus showed statistically significant choice responses after observing the intentional hand grasp and hand-occupied elbow touch actions, and responded at chance levels after observing the accidental hand flop and hand-empty elbow touch actions. A direct replication of the originally reported results on rhesus monkeys in Wood *et al.*, including the raw data, is available at www.sciencemag.org/cgi/content/full/317/5843/1402/DC2. Published online 25 April 2011.

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TECHNICAL COMMENT ABSTRACTS

Comment on "Erosion of Lizard Diversity by Climate Change and Altered Thermal Niches"

Susana Clusella-Trullas and Steven L. Chown

Using a regionally calibrated model, Sinervo *et al.* (Reports, 14 May 2010, p. 894) predicted potential climate change impacts on lizard populations and estimated

that many extinctions are under way. We argue that this model is not sufficient for predicting global losses in lizard species in response to anthropogenic climate change. Full text at www.sciencemag.org/cgi/content/full/332/6029/537-a

Response to Comment on "Erosion of Lizard Diversity by Climate Change and Altered Thermal Niches"

B. Sinervo, D. B. Miles, N. Martínez-Méndez, R. Lara-Resendiz, F. R. Méndez-De la Cruz

We derived physiological models that accurately predicted extinctions of Mexican and other lizards. Clusella-Trullas and Chown argue that global forecasts are unreliable without incorporating variance in microenvironmental temperatures, T_e . Here, we show that T_e variance is small relative to T_e increases from climate warming. Thus,

LIFE IN SCIENCE

Ichthyologists Hooked on Facebook

The Cuyuni River, which runs from Venezuela through Guyana and into the Atlantic via the Essequibo River, harbors hundreds of fish species. Although much of the river flows far from civilization, pollution from gold mining and other environmental hazards threaten its rich community of wildlife. Earlier this year, a small group of us from the United States and Guyana set out to perform the first comprehensive survey of the river's fishes with support from the Biological Diversity of the Guiana Shield program at the Smithsonian's National Museum of Natural History. We aimed to determine which species still thrived in the river, which might have disappeared, and whether any of the remote river's denizens were entirely new to science.

We arrived in January, during the dry season, and embarked for two weeks of collecting and camping along 200 km of rainforest-lined river. Local boatmen helped us navigate the often perilous river, and each day, we stood neck-deep in the muddy waters and pulled wide nets to catch samples of the life teeming beneath the surface. One student, Whit Bronaugh, photographed each species as the collection grew to hundreds, and then thousands, of fishes large and small.

Upon returning to Georgetown (Guyana's capital), a major challenge confronted us. As a condition to securing an export permit, we had just

EDITOR'S NOTE

This is an occasional feature highlighting some of the day-to-day humorous realities that face our readers. Can you top this? Submit your best stories at www.submit2science.org.

one week to complete a detailed report with each of our 5000 specimens identified to genus and species. Given the limited library resources at our disposal and the time constraint, the task seemed impossible.

Then one of the students, Devin Bloom, suggested posting



our many photographs on Facebook and inviting the ichthyologists among our circle of friends to help us identify them. Would that far-flung community take the time to help us? We decided to find out.

We posted the photographs (1), and within minutes comments began to pour in. "They look like fish to me" one commenter cheerfully acknowledged. On an anchovy, another noted, "Pizza topping." But before long, more insightful messages began to appear. On a catfish: "would say *Megalechis personata*," followed by another suggestion, "my guess would be *Megalechis thoracatum*." Amazed, we collected identifications from our friends, and then from friends of friends, contributing

their expertise from around the world. Less than 24 hours later, 90% of our specimens were identified. Armed with our export permit, we packed our specimens for shipment and returned home, grateful beyond words for the generosity of our colleagues, and for the social network that allowed us to harness their vast collective expertise and provide faster and more accurate identifications than we ever would have dreamed possible.

BRIAN SIDLAUSKAS,^{1,2*} CALVIN BERNARD,³ DEVIN BLOOM,⁴ WHITCOMB BRONAUGH,¹ MICHAEL CLEMENTSON,³ RICHARD P. VARI²

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Note

1. See the photographs at www.facebook.com/album.php?aid=243121&id=1049262482&l=e485413c15.

EVOLUTION

Laws of Cooperation

David Krakauer

The greatest challenge to science is to explain the prevalence of improbable, stable structure in the universe. Entropy mercilessly ensures that through time disorder is bound to increase. Discovering general mechanisms that favor local order is in some sense the most important game that all theorists in the natural sciences have been playing since the mid-19th century. In biology, theorists have focused on the battle between the dissipative tendencies of selfishness and the structuring capabilities of cooperation. Theoretical biologists—like theoretical physicists working in the abiotic domain before them—have sought natural laws that explain when and where cooperative order, and consequently adaptive complexity of various kinds, might come into existence.

In *SuperCooperators*, mathematical biologist Martin Nowak and *New Scientist* editor Roger Highfield present for nonspecialists recent progress in understanding how cooperation evolves from the competitive interactions inherent in Darwinian dynamics. They argue that we now know enough about the mathematics of adaptation to conceive of cooperation as a fundamental evolutionary principle and not merely as a surprising feature of biology in some way at odds with evolutionary processes.

We currently think of mutation, competition, and selection as fundamental properties of all evolutionary processes at different levels of explanation. Nowak and Highfield suggest that cooperation should be elevated to a similar status. They devote much of the book to building their case, presenting five candidate mechanisms (repetition, reputation, space, multilevel selection, and kin selection) that, when key inequalities are satisfied, allow cooperative configurations to outnumber selfish ones.

Many readers will turn to the book for further insights into the ongoing argument that pits Nowak and his colleagues against pro-

ponents of Hamiltonian kin selection. I have been rather perplexed about the source of so much uproar (beyond the obvious sociology of science and the desire that precedence be acknowledged), and the book should go some way toward clarifying the nature of the debate for outsiders (albeit with a participant's perspective). Two chapters, "Kin selection—nepotism" and "The lord of the ants," most explicitly address the dispute.

Nowak's essential (and rather simple) claim is that all forms of cooperation can be understood in terms of individual-level selection operating in hierarchically structured populations. The source of structure can be kinship, spatial constraints on mobility, or

selection. Nowak and colleagues, along with several population geneticists before them, suggest that we model individual selection and hierarchical population structure explicitly and do away with the phenomenological ambiguities of inclusive fitness arithmetic. I suspect that this is what will eventually happen, because theoretical science ultimately favors mathematical clarity and efficiency.

The book includes ideas that might reconcile the ground-breaking work of Hamilton and subsequent developments with the structured population dynamics offered by Nowak, Corina Tarnita, and Edward Wilson (1). Since the 1960s, when Hamilton wrote down his famous rule for the fixation of cooperates in terms of the ratio of benefits to costs and the coefficient of relatedness ($rb > c$), there has been a proliferation of analogous inequalities. Nowak describes several, including a graph theoretic version, $b/c > k$ (where k is the average number of nodes to which a focal node is connected in a network), and a group selection form, $b/c > 1 + n/g$ (where g is the number of groups and n the number of individuals per group). All of these recent inequalities explain the fixation of cooperation in terms of two classes of separable mechanisms: reproductive or growth terms (functions of time) and population structure terms (functions of space). Hamilton was the first to intuit the form of these evolutionary laws, and Nowak and colleagues have been pinning down their fundamental origins and meaning.

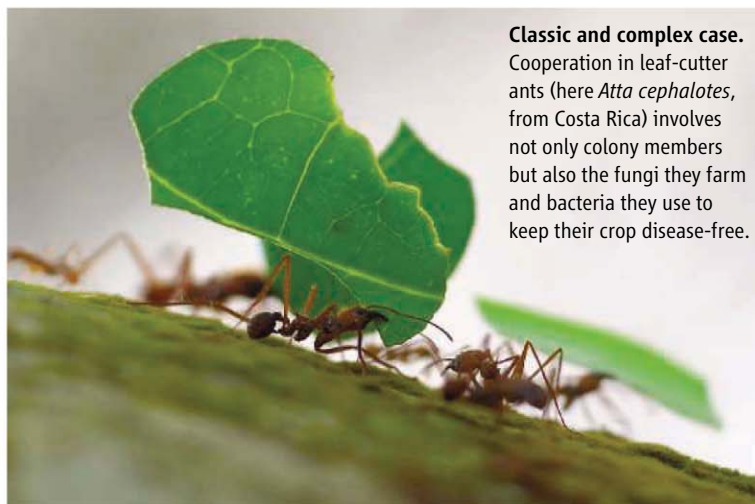
In the penultimate chapter, Nowak suggests unification in the form of one parameter, his sigma—the structure coefficient. Sigma measures the rate of like interactions in an evolutionary game (e.g., cooperators encountering cooperators or defectors encountering defectors). When sigma exceeds unity, cooperation invades; when below, selfishness. Sigma captures in one scalar value essential features of assortative interactions, and hence it points toward the essential processes underlying the origin of cooperation.

SuperCooperators

Altruism, Evolution, and Why We Need Each Other to Succeed/Evolution, Altruism and Human Behaviour or Why We Need Each Other to Succeed

by Martin A. Nowak, with Roger Highfield

Free Press, New York, 2011.
352 pp. \$27, C\$29.99.
ISBN 9781439100189.
Canongate, Edinburgh, 2011.
£20. ISBN 9781847673367.

**Classic and complex case.**

Cooperation in leaf-cutter ants (here *Atta cephalotes*, from Costa Rica) involves not only colony members but also the fungi they farm and bacteria they use to keep their crop disease-free.

mechanisms of individual recognition and attraction. Historically, kin selection has suffered from a few ambiguities. As originally conceived by Bill Hamilton, kinship implied genetic relatedness. It was soon recognized that this was too restrictive, and the approach was expanded to include multiple forms of assortativity. Hamilton also introduced the concept of inclusive fitness, which is where the substantive argument arises. Inclusive fitness calculations are often articulated in terms of coefficients of relatedness (not assortativity in general), which moreover only make sense under rather limited conditions of weak

I have little doubt that *SuperCooperators* will generate great interest and debate and meet with its fair share of dissension. Nowak, a very successful and plainspoken scientist, has a disinclination to compromise. His style has always been to forge ahead with alacrity, and at times this has led to an absence of historical considerations that offends those working on related problems. But he and Highfield should be commended for communicating in an accessible language many fascinating insights into the nature of cooperation. Their readers will find an informative survey of the rich mathematical models and theories that define a lawlike realm of biological and cultural phenomena.

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10.1126/science.1205650

POLITICAL SCIENCE

Dodging Responsibility

John Brehm

In *The Blame Game*, Christopher Hood identifies one of the most common gripes that citizens have about bureaucracy and government, namely, that no one in either accepts responsibility for making mistakes of omission or commission. In this brief and often illuminating book, Hood (a political scientist at Oxford University) explores the diverse and insidious ways in which ducking blame manifests in public life.

Hood's book follows in a spirit similar to other powerful approaches to politics by posing a single maximand (here, dodging blame) and inferring propositions that extend from this by-necessity unrealistic assumption. David Mayhew famously postulated that members of Congress must be single-minded seekers of reelection, for any other goal must be subordinate to seeking and retaining office (1). They may seek power, or have policy goals, or have aspirations for higher office, but all of these goals are for naught without election to office in the first place. With relevance to bureaucracy, Hood's blame dodging is akin to William Niskanen's budget-maximizing

bureaucrat, James March's slack-maximizing organization member, or Herbert Simon's satisficer (2–4). The weaknesses of such single-minded approaches are palpable: they require grotesquely simplified characterizations of the behavior of the actors (legislators, bureaucrats, organization members) they study.

Of course, all of these actors have other goals, some of which may provide better explanations of particular aspects of the actors' behavior. Yet the strength of single-minded approaches is that by providing clear assumptions about the maximands, authors can deduce specific, falsifiable propositions about behavior. Their methods of deduction might be formal (as with Niskanen), discursive (as with Mayhew), or a hybrid (as with Simon and March), but from each readers would learn to expect something: that the acquisition of a greater budget smotheres programmatic goals; that taking a position on controversial matters of politics matters less than claiming credit, which is in turn less frequent than simple advancement of name recognition; or that bureaucrats become less efficient in the long term by seeking resources that permit rapid response in the medium term.

What are the specific propositions that dodging blame implies? In his discussions, Hood identifies four main classes of actors: top leaders, street-level bureaucrats, intermediaries, and “all the different individuals with whom governments and public service providers deal.” (Hood has the habit of colloquializing many of the names of the actors: Top leaders are variously “generals” or “bananas,” and those directly serving the public are the “poor bloody infantry” or “frontline troops,” but none of these, often military, terms are really apt.) All these actors have a repertoire of ways to accomplish the same essential task: Leaders deflect blame toward subordinates, other agencies, or entities entirely outside of government; the front line shoves blame toward their leaders, other agencies, or outside entities; middle management might pass responsibility up, down, sideways, or out; “civil society” (the least coherent of the groups of actors) pushes sideways or outward. In short, all directions of blame shifting are possible—hardly the precision desired from a one-motivation model. Some of these directions are rather dysfunctional. Top leaders who blame subordinates may find themselves bereft of essential support in subsequent crises, and subordinates

who blame their chiefs may find themselves called on the carpet before too long.

Actors may avoid blame by ignoring present problems until public attention shifts elsewhere, persuading the public that the problem is not as bad as it seems (or could be), diverting attention to another problem, or even apologizing for the problem in the first place. One wonders what is left over, especially if apologies count as blame ducking—perhaps the degree to which the actor dodges blame has to do with the very subjective notion of how sincere the apology is? Hood approvingly mentions President Obama's apology and acceptance of blame after the failed attempt to appoint former senator Tom Daschle to the post of Secretary of Health and Human Services (“I screwed up”). Yet it is far from obvious that Obama had any particular role to play in the failed appointment other

than being in charge of an administration making its first appointment mistake.

The book contains multiple figures that resemble extensive form games and graphs displaying situations that suggest indifference curves, but Hood uses these graphical devices to illustrate rather than to press an argument. Nor does he provide much in the way of direct

quantitative evidence for his principal claims despite occasionally wishing for more useful indices. Instead, he supports his argument with qualitative evidence culled from stories of government or bureaucratic malfeasance. The anecdotes make for great story telling, but are they convincing data?

By the end of this short book, the reader may reach the rather unsatisfying conclusion that what we experience sporadically actually occurs regularly, for reasons that apply only occasionally. Bureaucracies may or may not be functional places where routine processing happens in a fair and appropriate way, but *The Blame Game* provides little guidance as to why politicians and government officials accept blame or how a polity might more effectively encourage appropriate responsibility.

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The Blame Game

Spin, Bureaucracy, and Self-Preservation in Government

by Christopher Hood

Princeton University Press,
Princeton, NJ, 2011.
238 pp. \$39.95, £27.95.
ISBN 9780691129952.

ETHICS

Ethical Framework for Biofuels

Alena Buyx^{1*} and Joyce Tait^{2,3}

Biofuels policies must protect human rights and the environment.

Biofuels made from renewable biomass are promoted as offering greenhouse gas (GHG) emissions savings and as one of the few options to replace liquid fossil fuels for transport. Mandatory targets for introduction and blending of biofuels have been introduced. For example, renewable fuels must account for 10% of transport fuel by 2020 in the European Union (EU) (1) and 36 billion gallons by 2022 in the United States (2). However, many claims that biofuels reduce GHG emissions have been contested (3, 4), and there are serious concerns about negative effects on food security, the environment, and the rights of farmers and landholders in developing countries (5–7).

Even though biofuels are only one part of the “energy mix,” and other sources raise other issues, it is nevertheless urgent to establish ethical conditions that biofuels development should meet and that should inform policy. We lay out briefly the main argument of the recent UK Nuffield Council on Bioethics’ report (8), concluding that many biofuels policies fail to satisfy ethical principles and recommending that they incorporate a number of ethical conditions, implemented through a certification scheme.

Ethical Framework

Despite vigorous debate, there has been little systematic ethical analysis and few efforts to develop policies that implement a more ethical approach to biofuel production. The problems of biofuels have often been discussed in a piecemeal way, but to avoid harm and reap potential benefits, ethical concerns should be part of an integrated analysis that gives a clear policy steer.

Using the idea of overlapping consensus (i.e., that differing, reasonable viewpoints share some common concerns) (9), the Nuffield report identifies moral values that are



Farmer harvests corn in Somaliland.

increasingly recognized in debates on global justice, climate change, and environmental ethics (10–12): the common good of mitigating climate change; respect for human rights; a commitment to solidarity with vulnerable populations; and notions of stewardship, sustainability, and intergenerational justice (8). To apply these values practically to biofuels development, the report authors derived an ethical framework that includes five principles that policy-makers can use to evaluate biofuel technologies and guide policy-making (8). Under each principle below, we give example policy recommendations.

1. *Biofuels development should not be at the expense of people’s essential rights.* People have the basic right to be protected from threats to their life, health and well-being, and ability to subsist (13). Biofuels targets have encouraged producers to scale up production rapidly, sometimes developing biofuels in countries with lax regulations (14). In some cases, this may have contributed to higher food prices or led to human rights abuses, for example, workers living in near-slavery conditions (5, 15). Target-based policies need to be more responsive to such unintended consequences and allow for changing the pace of up-scaling. Although presenting challenges of definition and agreement, compulsory certification schemes including human rights elements are necessary and could help ensure that all biofuels meet human rights standards (8).

2. *Biofuels should be environmentally sustainable.* The obligation to be responsible stewards of natural resources and ecosystems services for current and future generations is captured in the idea of environmental sustainability (16). In addition to biodiversity losses caused by land clearing and land-use change (both direct and indirect), biofuel crops can lead to water over-use and pollution through pesticide and fertilizer use. Policies related to biofuels’ sustainability are generally weak and vary from country to country. For example, the European Commission (EC) has suggested that each member state develops its own

standard, potentially leading to 27 different standards in the EU (17). A mandatory and enforceable international environmental sustainability standard for biofuel production, ideally developed by an international agency such as the UN Environment Programme, should replace such voluntary schemes.

3. *Biofuels should contribute to net reduction of total GHG emissions and not exacerbate global climate change.* Climate change is predicted to impose increasing harms, in particular on those most disadvantaged. Thus, climate change mitigation is a vital common good (8). Biofuels are expected to reduce GHG emissions. However, there is considerable uncertainty regarding measurement of emissions, and there are no controls to ensure that imported biofuels offer emissions savings throughout their production life cycle. A single international standard including a clear methodological framework for calculating GHG emissions across the whole biofuel life cycle would be the best way to ensure such savings. An ongoing initiative toward such internationalization of methodology is the Global Bioenergy Partnership (18). Moreover, a global, coordinated response to climate change from the international community should address land-use change directly, with strong international and local measures to prevent destruction of high-carbon stock. Although ambitious, this appears to have been recognized in Intergovernmental Panel on Climate Change debates after the 2010 Cancun summit. Practical, effective interven-

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tion on a global scale would make it unnecessary to develop separate policies to address indirect land-use change related only to biofuel development.

4. *Biofuels should recognize the rights of people to just reward.* Adequate payment for labor has been recognized as a basic human right (13) and is a central element of just reward (8). For example, the European Renewable Energy Directive requires that workers in all countries producing biofuels for the EU market are fairly paid and that working conditions are in accord with resolutions of the International Labor Organization (1). However, producers outside the EU may not abide by these policies, which can lead to overwork and low wages. Policy-makers should consider social impacts of mandated biofuels imports and implement strict requirements with strong audit trails for pay and working conditions that respect vulnerable populations. Although it needs improvement, a useful example is the voluntary Bonsucro scheme, which tries to improve labor rights in the sugarcane industry.

Intellectual property (IP), another important element of just reward, will play a key role in incenting investment in biofuels and securing profits for investors. Recognizing the distinction between existence and exercise of IP rights, the flexible use of license agreements, as advocated by the OECD with regard to genetic inventions (19) would support a better balance of parties' interests.

5. *Costs and benefits of biofuels should be distributed in an equitable way.* Short- and long-term costs and benefits of biofuel production extend well beyond financial losses or gains. There may be environmental, political, social, or economic benefits or burdens that apply only to certain sections of society, leading to questions of distributive justice (20). For example, investment in biofuels may threaten food security in poor countries, while delivering benefits for climate change and energy security in the developed world. Harms and benefits also depend on crop type and the land it is grown on, as these codetermine GHG emission savings or food versus fuel trade-offs.

Policies that govern biofuel production should balance needs of local and international markets and not disadvantage small-scale producers who could provide essential energy to the local community. Furthermore, access and benefit-sharing schemes, for example, as part of public-private partnerships, could be developed to encourage equitable distribution of costs and benefits. For emerging biofuels, a cost-benefit analysis at the proof-of-concept stage should take

account of the ethical principles proposed here. This should inform evaluation of policy options for large-scale implementation of all biofuels and for energy technologies more generally.

From Ethics to Policy

A new international approach to policy-making is required, guided by a transparent standard that reflects all areas of ethical concern discussed above; that satisfies requirements of procedural justice (i.e., fairness in the decision-making process); and that establishes minimum requirements for ethical biofuels production. Countries should be encouraged to exceed this standard, but its minimum requirements need to be implemented through an international, mandatory certification scheme. This should in turn allow for proportionate application, responsive to differences in circumstances in different countries, enabling local small-scale biofuel production, with some flexibility on the timescale of implementation, and financial support and advice for countries that might struggle to implement certification because of limited economic development. The Roundtable on Sustainable Biofuels recently proposed a standard and certification scheme with detailed guidelines, criteria, and indicators of compliance that could serve as a useful starting point (21). Introduction of such a system at national and regional (e.g., EU) levels would help establish a market in ethically produced biofuels.

An Ethical Duty to Develop Biofuels?

Efforts to reduce energy consumption are essential, but it is unrealistic to expect liquid fuel consumption to decrease over the next few decades (22). It is therefore important to develop ethical, sustainable alternatives to fossil fuels. In view of the common good of climate change mitigation and the need to reduce harm to humans and ecosystems, the Nuffield report concludes that there is an ethical duty to support development of new biofuels that can satisfy the five ethical principles (8), subject to absolute- and opportunity-cost considerations. Lignocellulosics and algae are candidate sources, but others may emerge.

However, there are currently few incentives for such innovations. Policy-makers could promote research and development of new biofuel technologies that meet the ethical principles, but this alone is unlikely to be sufficient to deliver necessary changes in practice. Widespread adoption of the proposed ethical standard and certification would create the appropriate policy and commercial environment to help ensure that improved,

ethically derived biofuels have the necessary market advantage over current biofuels. The ethical framework described here could also be applied to similar technologies and products; be part of comparative analyses of energy and climate-change abatement options; and be used to assess governance mechanisms, for example, revisions being considered for the Convention on Biodiversity and the Cartagena Protocol (23).

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Damping by Depletion

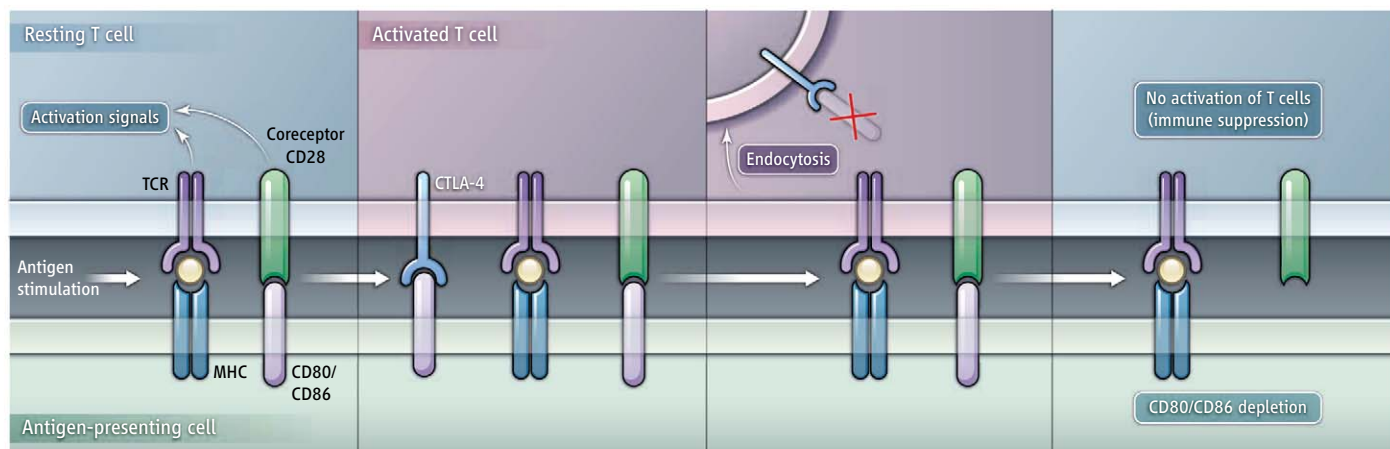
Shimon Sakaguchi¹ and Kajsia Wing²

Superfluous or misguided human immune responses may lead to harmful outcomes, as seen in autoimmune disease and allergy, but the immune system has evolved mechanisms to suppress these reactions. One of the key molecules engaged in such suppressive control is cytotoxic T lymphocyte antigen-4 (CTLA-4); also called CD152 (1). The molecule is expressed by certain T cell subpopulations, and animals lacking CTLA-4 die prematurely from mas-

both bind to the same ligands (CD80 and CD86) expressed on the surface of antigen-presenting cells (1). Following stimulation by antigen, CTLA-4 expression is induced in activated T cells such as helper T cells (CD4 subclass, which enhances the activity of other immune cells) and cytotoxic T cells (CD8 subclass, which induces the death of target cells, such as cancer cells). CTLA-4 is also constitutively expressed by regulatory T (T_{reg}) cells (a subpopulation of those

An immunoregulatory protein removes ligands from antigen-presenting cells, thereby suppressing immune responses.

the CTLA-4–CD86 complexes through endocytosis, and rapidly degrade CD86 molecules in the cytoplasm. The authors also show in vivo that, after administering antigen to normal animals, antigen-specific T_{reg} cells predominantly endocytose CD86 from antigen-presenting cells, whereas CTLA-4-deficient T_{reg} cells do not. CD80 seems to be similarly removed from the surface of antigen-presenting cells because it has a higher affinity for CTLA-4 than does CD86. Thus, depletion of



Snatched. A naïve T cell (or T_{reg} cell) expresses CTLA-4 when activated by signals from the T cell receptor (TCR) and coreceptor CD28. The receptors interact with antigen and CD80 (or CD86), respectively, on an antigen-presenting cell. The CTLA-4–CD80

(or CTLA-4–CD86) complex is endocytosed by the activated T cell, depleting CD80 (or CD86) from the antigen-presenting cell. The depleted cell cannot further activate the T cell or other T cells. MHC, major histocompatibility complex.

sive T cell proliferation and severe autoimmunity (2–4). It has not been clear how CTLA-4 negatively controls immune responses via these T cells, but on page 600 of this issue, Qureshi *et al.* (5) report a mechanism by which the molecule modulates the function of antigen-presenting cells (such as dendritic cells) to control immunological self-tolerance and homeostasis.

Activation of a naïve T cell by antigenic stimulation requires two independent signals: one from the T cell receptor upon its association with antigen presented to it and the other from the costimulatory receptor CD28, both in the immunological synapse (the interface between an antigen-presenting cell and a T cell). CD28 and CTLA-4 are structurally homologous, and

that express CD4), which are specialized for immunosuppressive function (6).

For more than two decades, CTLA-4 has been considered a co-inhibitory receptor, transducing a negative signal into an activated T cell that attenuates T cell receptor and CD28 signals. In support of this idea, ligation of CTLA-4 molecules on activated T cells by specific antibody inhibits their production of interleukin-2, a major cytokine required for T cell proliferation, differentiation, and survival (7, 8). Yet, CTLA-4 may also have a “cell-extrinsic” function in immune suppression (4, 9). That is, CTLA-4 expressed by T_{reg} cells and activated T cells dampens the expression of CD80 and CD86 by antigen-presenting cells.

Qureshi *et al.* demonstrate that when CTLA-4-expressing antigen-activated T cells are cultured with CD86-expressing dendritic cells, the former remove CTLA-4-bound CD86 molecules from the surface of the dendritic cells. T cells actively internalize

CD80 or CD86 from antigen-presenting cells by CTLA-4-dependent trans-endocytosis, and the resulting inability of the antigen-presenting cell to provide T cells with costimulation through CD28, inhibits excessive activation of CTLA-4-expressing T cells and may also allow T_{reg} cells to suppress the activation of other T cells recruited to the antigen-presenting cells (see the figure).

Yet, the findings of Qureshi *et al.* do not exclude other possible cell-extrinsic or cell-intrinsic functions of CTLA-4 suggested by the variant forms of the protein, including ligand-dependent and ligand-independent forms, as well as the soluble one (1). In addition to its role in trans-endocytosis, CTLA-4 may simply outcompete CD28 for binding to CD80 or CD86 in the immunological synapse because of its much higher binding affinity for the ligands and its increased expression upon T cell receptor stimulation (10). Soluble CTLA-4 might block CD80 and CD86 (1). The ligand-independent form may attenuate

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T cell activation by a cell-intrinsic mechanism (11). CTLA-4 may also transduce signals via CD80 and CD86 into antigen-presenting cells and induce their production of an immunosuppressive metabolite, repress the transcription of the genes encoding CD80 or CD86, or inhibit the production of inflammatory cytokines, although how T cells evoke these events in antigen-presenting cells via CTLA-4 remains obscure (12).

CTLA-4 is a key molecular target for enhancing or damping immune responses clinically. CTLA-4 blockade by specific monoclonal antibody evokes effective

immune response to tumors, and blockade of CD80 or CD86 by a CTLA-4-immunoglobulin fusion protein suppresses autoimmune responses and establishes transplantation tolerance (13, 14). Assuming that CTLA-4 has multiple immune inhibitory roles, including trans-endocytosis of its ligands, further study of the cell-extrinsic and cell-intrinsic functions of this molecule will enable better control of physiological and pathological immune responses.

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APPLIED PHYSICS

Phase-Change Memories on a Diet

Martin Salinga and Matthias Wuttig

In 1637 René Descartes was pondering the possibilities of human-made machines, being certain that their potential would be very limited. It was inconceivable to him that a machine could ever “arrange words so as to adequately reply to what is said in its presence” (1). The recent competition between IBM’s supercomputer Watson and two of the most successful *Jeopardy* champions demonstrates how far human-made machines have progressed. Nevertheless, Watson’s success comes at appreciable cost: Whereas humans perform their daily duty of “thinking” by con-

suming an average power of only 50 W, Watson consumes more than 2 MW. Imagine how helpful computers could be if processing and storing data could be accomplished by much less power-hungry computers. On page 568 of this issue, Xiong *et al.* (2) demonstrate a remarkable step forward whereby a new design for phase-change memory can lead to a drastic reduction in the power requirements for its operation.

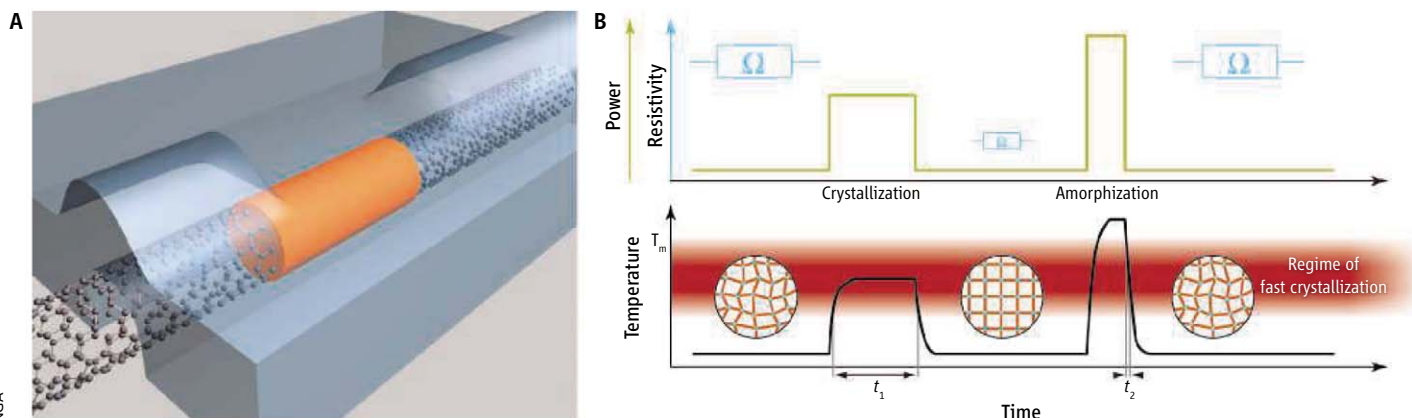
In phase-change materials, a short voltage pulse is used to heat the crystalline phase above the melting temperature; rapidly quenching the liquid creates an amorphous state with higher resistivity. Applying a second pulse with lower voltage switches the phase-change material back to the crystalline

Device design helps reduce the power requirements for switching phase-change memory.

state, hence enabling rewritable data storage (3) (see the figure, panel B). This storage principle is nonvolatile—the information is not lost when the power to the storage medium is turned off. Because phase-change memories can also be very fast, comparable to the speed of volatile dynamic random access memories, they might provide the storage attributes of a universal memory, replacing several current memory technologies at once.

With greater demands to reduce the power needed to store and retrieve data, emphasis increasingly shifts to reducing the necessary pulse power to store data. Several approaches can reach this goal in phase-change memories. A particularly promising one is the reduction in the active volume of phase-change mate-

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Memory switch. (A) A gap in a carbon nanotube lying on a substrate is filled by depositing a film of phase-change material (transparent blue) on top of the nanotube and its surrounding environment. The application of short current pulses switches only the well-defined volume in the gap of the nanotube. A large reduction in the active volume (orange cylinder) and especially of its contact area is achieved. (B) When the amorphous material is heated by an electrical

current, its atoms become mobilized enough to find their way into the energetically more favorable crystalline state. The amorphization of a previously crystalline volume is obtained by heating above the melting temperature T_m and subsequent rapid cooling of the material, minimizing the time t_2 at temperatures that cause fast crystallization. The strong contrast in resistivity between the phases allows for an electrical determination of a cell’s state.

rial (4, 5). Previous work has already shown that it is possible to scale phase-change memory cells down to the sub-20-nm technology node (6). The cross-sectional area of those cells, however, still exceeds 400 nm².

Xiong *et al.* investigate the power necessary to switch a region of phase-change material with a cross-sectional area of just 7 nm² with a clever trick of using extremely thin carbon nanotubes as electrodes. To build a phase-change memory cell, they briefly apply a high voltage to the ends of a carbon nanotube, which creates a gap in the nanotube, a few tens of nanometers wide. This gap is then filled by depositing a film of phase-change material on top of the nanotube and its surrounding environment. The subsequent application of short current pulses switches only the well-defined volume in the gap of the nanotube (orange cylinder in the figure, panel A).

With this approach, Xiong *et al.* investigate how the power necessary to store data in phase-change materials scales with the lateral extension of the region to be switched. The current necessary to amorphize (reset) a memory cell with a 7-nm² contact area is around 5 μ A. This is at least one order of magnitude lower than the current required

to switch more conventional memory cells with a contact area of around 400 nm². This remarkably low value demonstrates the excellent scaling potential of phase-change memories. But how much further can the contact area be reduced until a fundamental limit is approached? The work by Xiong *et al.* already pushes the cell size limit to 7 nm². Given the present speed of improvement in the semiconductor industry, it will take more than a decade until commercial cells will reach this size, extending the scaling potential of electronic data storage considerably.

Although the present approach focuses on the optimization of memory cells by reducing their volume, it should also be rewarding to improve phase-change materials further. Identifying materials with a relatively low melting temperature would be useful, as such materials need less power to be amorphized. Identifying materials with even lower thermal conductivity would ensure that little power is wasted to heat the surrounding region. Finally, materials that switch more rapidly would be desirable (7, 8), as a reduction in the duration of applied electrical pulses will decrease the energy per switching event.

The results of Xiong *et al.* could help a future Watson to have less appetite for energy. Possibly this successor of Watson might even incorporate phase-change materials for a different reason. The ability to program variable resistance levels makes phase-change materials attractive for the development of neuromorphic hardware, a new class of hardware based on a computing logic more closely resembling the human brain. This strategy might bring humankind another step further toward a machine far beyond Descartes' imagination.

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BIOCHEMISTRY

The Two Faces of SAM

JoAnne Stubbe

The word “radical” (1) is often associated with highly energetic species (atoms or molecules) that chemically react with, and modify or destroy, molecules that they encounter. All organisms devote considerable metabolic effort to “control” radicals, such as reactive oxygen and nitrogen species (for example, the hydroxyl radical and nitric oxide), and to repair the damage that they inflict on macromolecules and small molecules alike. Radical-based reactions, however, also play an important role in metabolism. Evolution has produced mechanisms that harness radicals' reactivity to carry out difficult chemical transformations with exquisite specificity. For instance, while the common cellular compound called S-adenosylmethionine (SAM) is well known to play a role in the methylation of nucleophiles by a typical substitution reaction, in

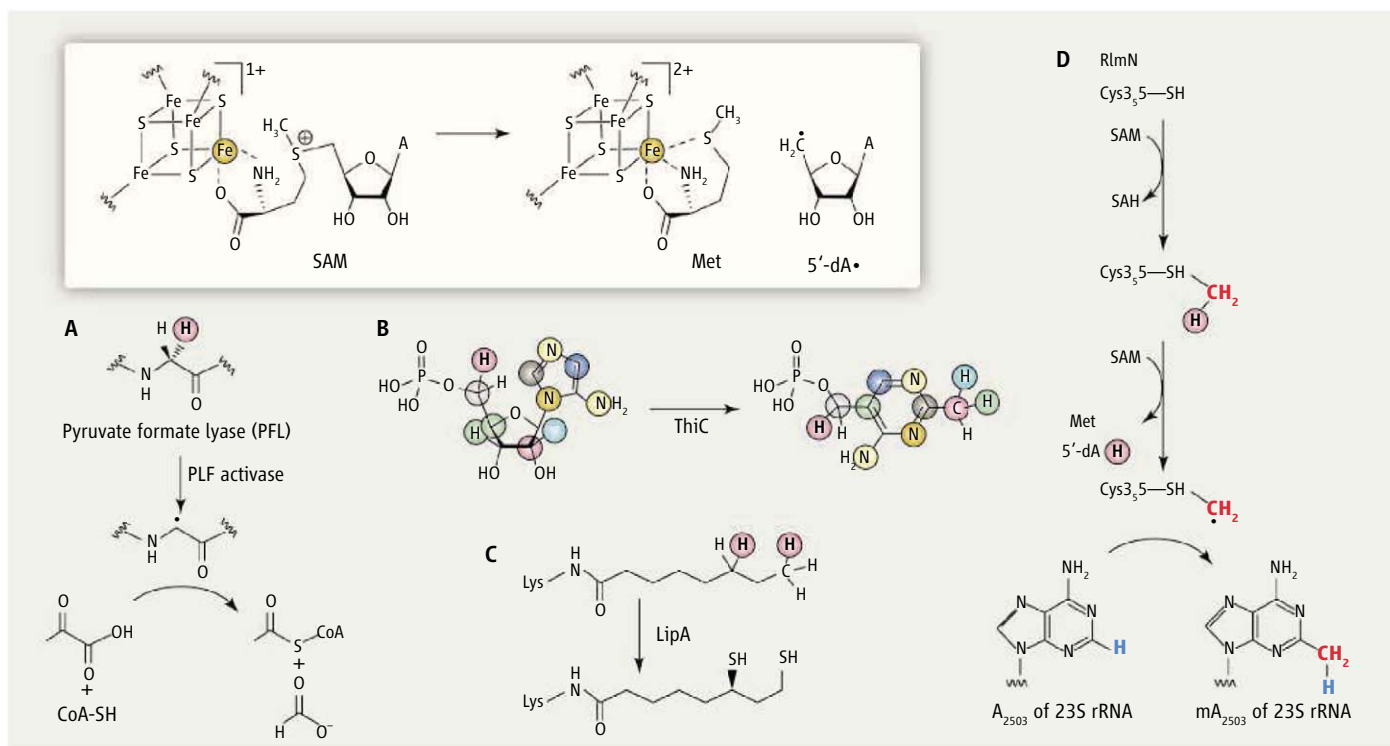
seminal work, Knappe and Schmitt (2) demonstrated that SAM mediates radical chemistry in organisms grown anaerobically (see the figure). Since then, investigators have implicated SAM in many unusual and diverse radical-based transformations.

On page 604 of this issue, Grove *et al.* (3) add to the list. They propose that SAM plays a central role in a novel radical chemical reaction that is involved in the development of antibiotic resistance in bacteria. They describe the unusual methylation of the C-2 and C-8 positions of an adenine (A2503) of the 23S rRNA (rRNA), catalyzed by two “methyltransferases,” RlmN and Cfr. Both enzymes require two SAMs to transfer a single methyl group. These reactions appear to involve a typical nucleophilic methylation of a conserved cysteine (Cys₃₅₅-SCH₃ in RlmN) in each protein by SAM, followed by SAM-dependent radical chemistry that generates an unprecedented protein radical on the methylated cysteine (Cys₃₅₅-SCH₂•). In *Science Express*, Boal *et al.* (4) provide strong evi-

An unusual radical mechanism enables ribosome methylation in bacteria.

dence for this proposal, presenting structures of RlmN involved in the reaction in the presence and absence of SAM.

The RlmN- and Cfr-catalyzed methylations described by Grove *et al.* add to the many novel reactions that involve radical chemistry and SAM (see the figure). In 2001, bioinformatic analyses by Sofia *et al.* (5) identified a new superfamily of enzymes, which they designated “radical SAM,” whose members shared a CX₃CX₂C motif and SAM as a cofactor. There are now proposed to be >3000 members (6, 7) of this family, including RlmN and Cfr. The family members share a requirement for a [4Fe-4S]^{2+/1+} cluster with a unique Fe that binds SAM in a bidentate fashion through its α -amino and carboxylate groups (see the figure) (8). Each reaction involves the reductive cleavage of SAM to generate methionine (Met) and a 5'-deoxyadenosine radical (5'-dA•), the species responsible for initiation of the radical-dependent reactions. The 5'-dA• is used to remove a hydrogen atom (H•) (red spheres



SAM's many roles. The $[4\text{Fe-4S}]^{1+}$ cluster plays a role in reductive cleavage of SAM to generate the 5'-dA• (box), which then functions to remove a hydrogen atom from a small molecule, protein, RNA, or DNA substrate and initiate complex radical chemistry. A number of examples are presented: (A) Glycyl radical formation in the polypeptide backbone of the anaerobic pyruvate formate lyase that catalyzes the conversion of pyruvate to acetyl-CoA and formate (2). (B)

Biosynthesis of thiamin, essential for transformations in pyruvate dehydrogenase and the pentose phosphate pathway (13). (C) Biosynthesis of biotin (14) and lipoic acids (15) via radical SAM enzymes that insert sulfur into unactivated C-H bonds. (D) Methylation of A2503 of the 23S rRNA of the 50S subunit of the ribosome by Cfr and RlmN (3, 10). The red spheres indicate the initial site of hydrogen atom abstraction.

in the figure) from the enzyme's substrate which can be a small molecule, DNA, RNA, or protein. After this initial step, the mechanisms involve transient radical intermediates unique to each enzyme.

Researchers are interested in Cfr and RlmN because they play a role in antibiotic resistance. Methylation at C-8 of A2503-23S-rRNA located in the peptide bond-forming site (A site) of the 50S subunit of the ribosome (9) confers resistance to five classes of antibiotics. Yan *et al.* (10) established that the enzyme's substrate is the "naked" 23S rRNA and that smaller RNA fragments can be methylated. Grove *et al.* established that an RNA oligomer as small as seven nucleotides can be methylated. Beautiful labeling studies, using trideuterated-methyl (CD_3)-SAM and CD_3 -methyl Cys_{355} of RlmN (CD_3 -RlmN) and Cfr, demonstrated their importance in the overall reaction and the requirement for two SAMs (see the figure). Unusual labeling patterns gave rise to Grove *et al.*'s proposal for the unprecedented mechanism underlying methylation. Very recently, studies by Yan and Fujimori (11) with CD_3 -SAM and C-2 deuterated A2503-rRNA gave labeling distributions consistent with those of Grove *et al.* (see

the figure). The proposals from the two labs for the mechanism underlying methylation however, are distinct. The structure reported by Boal *et al.* reveals the methylated cysteine ($\text{Cys}_{355}\text{-SCH}_3$) identified by Grove *et al.* (12) and provides strong support for the Grove *et al.* proposal that disulfide bond formation plays a role in product release.

These studies demonstrate the challenges associated with studying radical SAM enzymes. First, the essential iron sulfur clusters are oxygen-labile and, despite the universality of their biosynthetic pathways, cluster assembly often remains problematic in heterologous expression systems. Fully active enzymes are challenging to obtain. Second, the mechanisms of many of these radical SAM proteins lack known precedent and will be challenging to unravel. In the case of the RlmN (Cfr) mechanism, for instance, it remains to be established whether the $[4\text{Fe-4S}]$ cluster serves as a reductant and an oxidant, whether Cys_{355} is methylated by RlmN(Cfr) or by an additional protein, and whether the protein catalyzes multiple turnovers.

The studies on these methyltransferases, and similar studies on other systems, highlight the prevalence of radical-dependent

reactions in biological systems. It is up to chemists to identify the substrates for these enzymes and unravel the complexity of these fascinating reactions.

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PHYSICS

How Do You Want That Insulator?

Gregory A. Fiete

An expert chef can take ordinary foods and bring out extraordinary flavors and textures, usually through a combination of the right ingredients and exacting cooking techniques. If the electrical conductivity of a material can be thought of as the flavor of a dish, nature can serve up specialties, such as ceramic insulators that become superconductors when served cold and when the ingredients (the chemical components) are carefully tuned. Superconductivity is an example of an emergent quantum phenomenon—one that is created by the coordinated motion of many particles (1). On page 560 of this issue, Xu *et al.* (2) report how tuning the composition of normal insulators can turn them into topological insulators, which are another example of materials exhibiting emergent quantum phenomena (3–5). This result has important implications for the promise of topological insulators in lower-power devices that rely on electron spin rather than charge.

Most emergent quantum phenomena in condensed-matter physics require interactions among electrons, such as the pairing interaction between electrons in a superconductor. By contrast, the key ingredient for creating the topological insulator phase is spin-orbit coupling. Electrons have a quantum property known as spin that causes them to act in some respects like tiny bar magnets. Spin-orbit coupling forces a particular relation between the orientation of the spin and the orbital motion of the electron in space. In materials composed of light atoms, spin-orbit coupling effects are small and can be neglected, but when heavy atoms are present, their high nuclear charge can lead to appreciable magnetic fields in the frame of reference of the moving

electron. This field couples to the spin of the electron and can drive a phase transition to the topological insulator state (3–5). Mathematically, the difference between a normal insulator and a topological one can be described

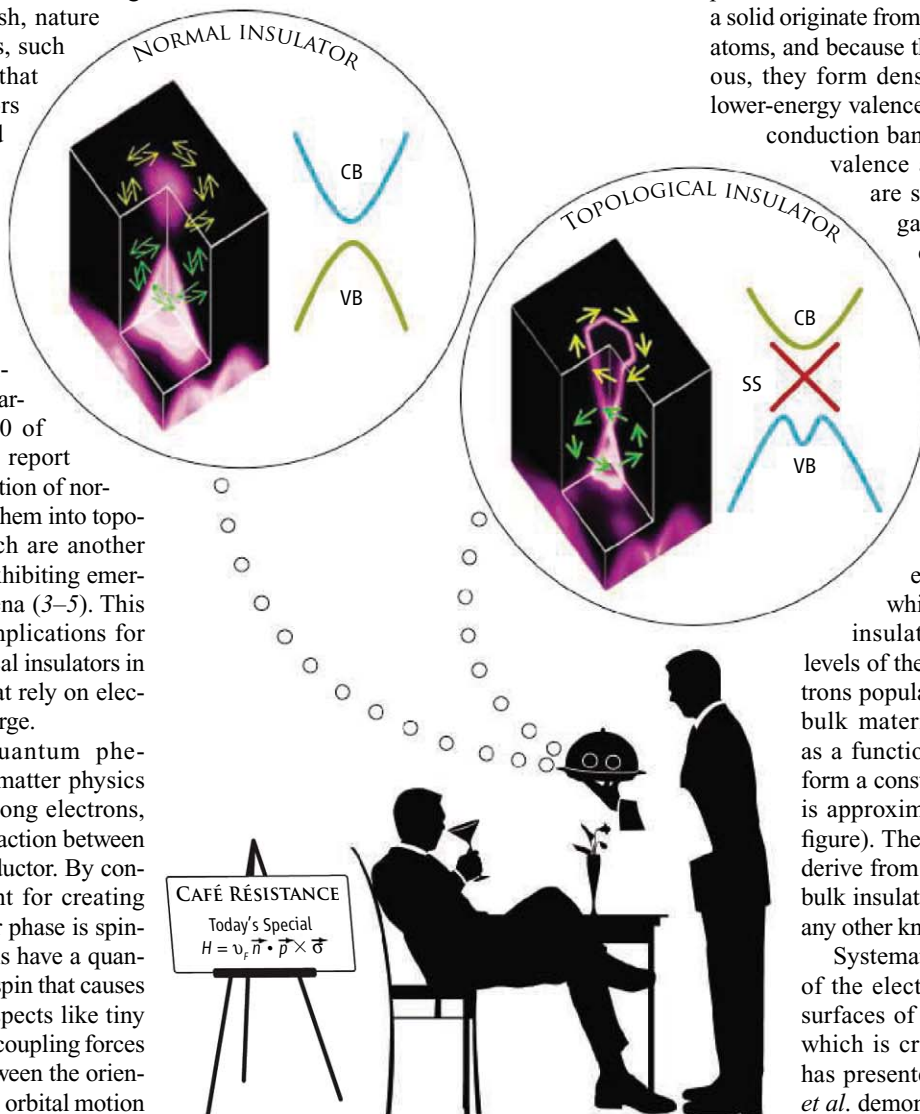
A normal insulator is turned into an exotic topological insulator by tuning its elemental composition.

with ideas borrowed from topology, hence the name topological insulator.

To appreciate the unusual nature of topological insulators, it is useful to see what causes a normal insulator (a ceramic) to be a poor conductor. The electron energy levels of a solid originate from those of the constituent atoms, and because the atoms are so numerous, they form dense bands of states: the lower-energy valence and the higher-energy conduction bands. In an insulator, the valence and conduction bands are separated by an energy gap, and the zero of the energy, known as the Fermi energy, lies in this gap where there are no conducting states (see the figure).

The unusual properties of topological insulators manifest at their surface, where additional states emerge that allow electrons to be mobile, while the bulk remains an insulator (3–5). The energy levels of these mobile surface electrons populate the band gap of the bulk material, and when plotted as a function of momentum, they form a constant-energy surface that is approximately circular (see the figure). The novel surface features derive from the composition of the bulk insulator and are not found in any other known system in nature.

Systematic and reliable control of the electrical properties of the surfaces of topological insulators, which is critical for applications, has presented a challenge (6). Xu *et al.* demonstrate a transition from a normal to a topological insulator by changing its bulk composition—replacing lighter sulfur (S) with heavier selenium (Se) to increase spin-orbit coupling. The material they studied, $\text{BiTl}(\text{S}_{1-x}\text{Se}_x)_2$ (where Bi is bismuth and Tl is thallium), starts to display a transition to topological insulator behavior at 40% Se, which fully develops by 60%.



Insulators made to order. The choice at the Café Resistance is between an ordinary insulator on the left and the more exotic topological insulator on the right. Xu *et al.* show that both properties can be tuned into the material $\text{BiTl}(\text{S}_{1-x}\text{Se}_x)_2$ by varying the fraction of sulfur and selenium. The normal insulator (sulfur-rich) has a band gap in the surface states, whereas the topological insulator (selenium-rich) has topologically protected metallic surface states running between the conduction and valence bands that cross at the Dirac point. The spin direction relative to the momentum changes sign for states above and below the Dirac point and is referred to as “texture inversion” by the authors.

To visualize this transition, the authors used spin- and angle-resolved photoemission spectroscopy to measure the spin and electron energy as a function of electron momentum. The replacement of S with Se creates metallic surface states with a linear energy-momentum relation. These surface extensions of the conduction and valence bands touch at a single node, called the Dirac point. The authors also observed subtle changes in the crystal lattice with x-ray scattering data. First-principles electronic structure calculations suggest that the transition depends on both the lattice changes and enhanced spin-orbit coupling.

Xu *et al.* take a further important step toward tuning the properties of the topological insulator by dosing a molecule, NO₂, on the surface that allows a tuning of the Fermi energy of the surface states. This step leads to a “texture inversion” of the spin-momentum relations when the Fermi energy passes through the Dirac point, as illustrated in the figure. Most of the novel electric and magnetic responses of topological insulators known to date rely on this tuning of the Fermi energy near the Dirac point (7, 8). A theoretical proposal for a “topological excitonic condensate,” an unusual symmetry-broken state with fractional charges ($\pm e/2$,

where e is the charge of the electron) that could be formed by topological insulators, may also be a step closer with this new experimental technology (9).

Despite the experimental accomplishments reported by Xu *et al.*, there are many challenges that remain if topological insulators are to become functional components of electronic devices. Chief among them are the problems of “aging”—the material properties degrade on a time scale of hours to days. Also, the bulk conductivity is unacceptably high and greater than what theory has predicted. However, there are good reasons to hope for substantial improvements in sample quality. The material Bi₂Te₂Se (where Tl is replaced by tellurium) was recently shown (10) to have a more insulating bulk, with up to 70% of the electrical conductance coming from the surface—more than two orders of magnitude better than most topological “insulators.” Parallel methods of sample fabrication—chemical synthesis and molecular beam epitaxy—are expected to continue to lead to sample improvements in the near future.

Thus far, the known topological insulators are derived from materials with s- and p-type orbitals (which typically have weak electron correlations), and experiment has largely operated in the mode of confirming theoretic-

cal predictions. A new frontier with experimental surprises likely lies in the direction of more strongly correlated materials with d- and f-type electrons (11–16), which should expand the exciting choices already on the insulator menu.

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MICROBIOLOGY

Alternative Actions for Antibiotics

William Croft Ratcliff and Robert Ford Denison

Microbes generate signals, which coordinate mutually beneficial activities (1). They also produce antibiotics that kill prey, suppress competitors, or deter predators (2). Recent observations have led to the view that antibiotics often act as mutually beneficial signals (3–6). Exposure to sublethal concentrations of antibiotics can indeed alter microbial metabolism and even change behavior in beneficial ways, triggering reactions such as fleeing or hiding within the protective environment of a microbial aggregate (biofilm). But the weapon-signal dichotomy of functions for these compounds is a false one—there may be other possible information-related actions of naturally produced antibiotics: cues and manipulation.

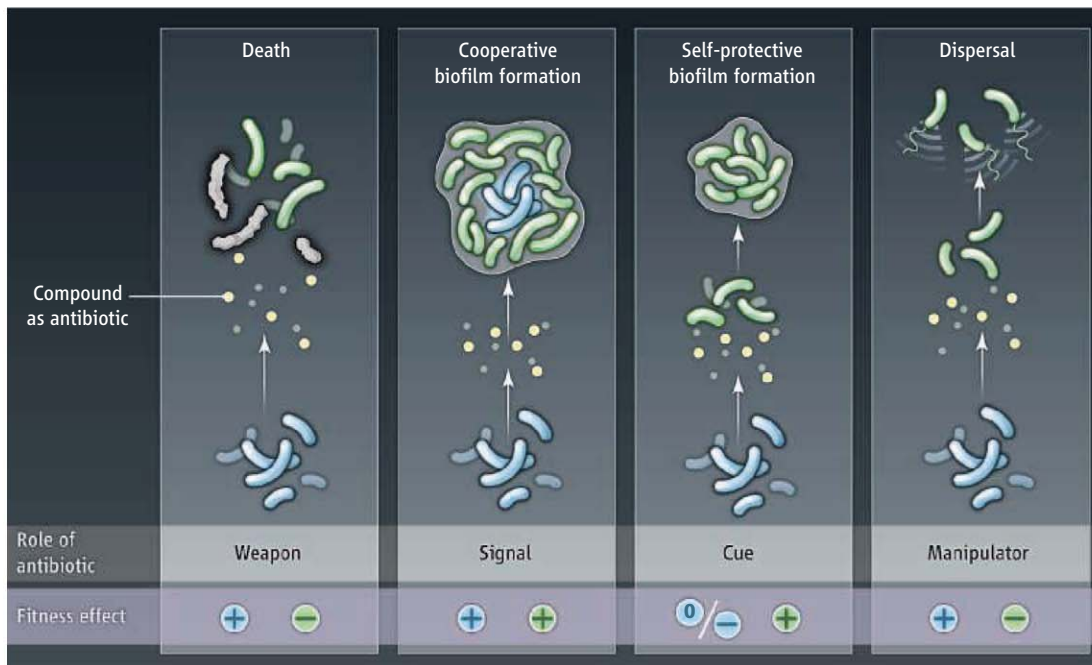
The antibiotic-as-beneficial-signal hypothesis proposes that in nature, antibi-

otics evolved as a means of communication between unrelated species of microbes, but cause death in the laboratory as a result of unnaturally high cell densities and antibiotic concentrations (4–6). Evolutionary theory, however, predicts that bona fide signaling between different species will be rare (7). That is, if producing metabolically costly signaling molecules aids a recipient without preferentially benefiting the sender, then it is a form of altruism and is unlikely to persist evolutionarily. By contrast, individually costly signaling can evolve among relatives through kin selection, which favors the reproductive success of an organism's relatives, even at individual cost (8). However, altruism toward another species is more difficult to explain. Evolutionarily stable between-species signaling would require a shared interest, such that both sender and receiver benefit as a result of the communication. Such shared interests are rare among species competing

Compounds recognized as having antibiotic functions may have other possible roles in microbial interactions.

for the same limited resources. Reflecting the stringent conditions required for its evolution, mutually beneficial signaling between animal species is much less common than signaling within species (7).

Beneficial signaling, however, is not the only possible alternative function for compounds with antibiotic (lethal) effects. Microbes detecting a low concentration of an antibiotic may interpret the compound as a cue that enables them to predict future exposures to an increased concentration. This cue allows them to respond in ways that reduce their susceptibility. For example, the bacterium *Pseudomonas aeruginosa* responds to sublethal concentrations of the antibiotic tetracycline by forming biofilms (5), thereby reducing future exposure to antibiotics (9), much as an animal joining a herd reduces its exposure to predation. Because joining a biofilm reduces the efficacy of the antibiotic, this action benefits the exposed microbe, with-



Naturally produced antibiotics. At high concentrations, microbially produced antibiotics can damage or kill other microbes. At low concentrations, these compounds can elicit changes in the behavior of other microbes. These alternative functions can be determined by examining the fitness consequence of producing the compound.

out preferentially benefiting the producer as would be required to qualify as a signal. Consistent with the hypothesis that antibiotics can act as cues, metabolic by-products generated by producer microbes, such as cell wall fragments and DNA, clearly not released to transmit information, can also induce behavioral changes that may benefit exposed recipient microbes (10).

It is also possible that compounds with antibiotic effects act as manipulating factors. For example, some microbes manipulate others by mimicking within-species signals used to coordinate group-level behaviors such as virulence, sporulation, or biofilm formation (1). This can trick the exposed microbes into adopting maladaptive physiological states or behaviors. For example, exposure to the antibiotic tobramycin causes *P. aeruginosa* to increase motility and flee (5). Consequently, competition for resources by the microbe that produces the antibiotic is reduced. Some acts of manipulation are quite sophisticated: Social amoebae in the genus *Dictyostelium* gather by the thousands when starved and cooperatively sporulate. *D. caveatum* has evolved to enter an aggregate of *D. discoideum*, produce a small molecule that inhibits sporulation, and then consume the thwarted *D. discoideum* (11). In this case, the small molecule does not function in a direct lethal manner; nor does it act as a signal because only the producer benefits from this communication. Moreover, manipulation is not

limited to microbe-microbe interactions, but can cross biological domains. The nitrogen-fixing bacterium *Bradyrhizobium elkanii* produces rhizobitoxine, a chemical that inhibits its legume host's ability to make the hormone ethylene, reducing plant growth but increasing the bacterium's resource acquisition (12).

Whether a microbial compound acts as a weapon, signal, cue, or manipulator depends on the fitness consequences of the interaction (see the figure). Signals benefit both the sender and recipient, cues benefit the recipient but not the sender, and manipulation benefits the sender but hurts the recipient (8). Weapons, like manipulation, benefit the producer at the expense of the recipient, but accomplish this by causing death or injury instead of inducing maladaptive behavior. To determine the function of these compounds in nature, these fitness consequences must be measured in the environment in which the microbes evolved.

If microbes of many different species were altruists and in constant communication among each other for mutual benefit, then it would follow that antibiotics may act as "collective regulators of the homeostasis of microbial communities" (5). But this argument has little evolutionary merit: Heritable variation among communities is small relative to that within communities, partly because individuals migrate among communities. Individual and kin selection, therefore, swamp community-level selection, and

individuals never evolve mechanisms for the benefit of the community as a whole (13). Similarly putative signals released by human gut microbes to mediate beneficial outcomes for both microbes and humans (14) may actually represent manipulation or inadvertent cues, respectively, depending on whether they decrease or increase the host's immune responses.

In examining the roles played by antibiotics, we should not ignore the possibility of multiple functions. Molecules classified as antibiotics may indeed be used as signals to coordinate behavior among related microbes. Research that unravels the specifics of this communication will improve

our understanding of microbial evolution and ecology and may lead to new treatments for microbial infection. For example, bacterial biofilms are a major contributor to the persistence of microbial infections in humans. These may be treated by interfering with signals required to form biofilms (15) or by deliberately providing signals coordinating dispersal (16), both examples of human-mediated manipulation.

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RETROSPECTIVE

Thomas Eisner (1929–2011)

Jerrold Meinwald

With the passing of Thomas Eisner on 25 March 2011, at the age of 81, the world has lost one of its most original and influential scientists. We owe the development of the contemporary discipline of chemical ecology largely to him. Eisner was fascinated by insects throughout his childhood. Wherever he found himself, he would observe in meticulous detail the behaviors of his favorite “bugs,” carefully noticing how these marvelously diverse creatures managed their interactions with one another and with their environment. He maintained a lifelong curiosity and regard for insects and their chemical treasures.

Born on 25 June 1929 in Berlin, Eisner moved with his parents to Barcelona, Spain at the age of 4, and after a brief stay in Paris, moved to Montevideo, Uruguay. In 1947, the family moved to the United States, and Tom attended Champlain College before transferring to Harvard University. By the time he completed his undergraduate and graduate studies at Harvard (where he earned a Ph.D. in biology), he had developed into one of the greatest naturalists of his generation. Stimulated by Harvard faculty members, and perhaps even more by extensive contact with fellow students—among them Edward O. Wilson, Julius Adler, and Eric Kandell—Eisner’s love and knowledge of nature continued to grow throughout his more than half-century career in science.

Tom Eisner joined the Cornell University faculty in 1957 as an assistant professor in the Department of Entomology. Howard Schneiderman, a mutual friend in Cornell’s Department of Zoology, introduced us to one another shortly after Tom’s arrival, saying something like “This is my good friend, Thomas Eisner, who has just come to Cornell. He is interested in all sorts of insect-related projects, and some of them might benefit from chemical collaboration.” It was the beginning of our eating lunches together in the Faculty Club, where he would pour forth a string of stories about defensive techniques



practiced by a wide variety of arthropod species, including whip scorpions, daddy long-legs, millipedes, centipedes, ants, beetles, and caterpillars of all sorts. It turned out that many of these stories did indeed involve unexplored chemistry. Our joint endeavors to understand the lives of arthropods at the molecular level occupied not only the two of us but also our research students for the next five decades. What was unique about Tom was his ability to bring his subjects into the laboratory, to develop quantitative bioassays, and, when the problem at hand required, recruit the appropriate chemical, physical, or engineering collaborators to gain a deep understanding of his initial field observations. Tom’s work did not go unnoticed: Just a dozen years after joining the Cornell faculty, at age 40, he was elected to membership in both the American Academy of Arts and Sciences and the National Academy of Sciences.

Tom Eisner took the greatest care in writing his scientific papers. We wrote many of these together, and he could be counted on to think of a clearer and more elegant way of stating our case than I had been able to do alone. His command of spoken languages was also remarkable. He was fluent in German, Spanish, and French, and although he was 17 when he came to the United States from Uruguay, he soon spoke absolutely accent-free English. His lectures were packed full of intriguing information and insights. They were fast-paced, witty, and beautifully organized. They

A biologist who marveled at insects and their arsenal of compounds sparked the field of chemical ecology.

transmitted his unmistakable enthusiasm for his subject perfectly, and it is no wonder that he inspired so many of his audience members.

Eisner’s skill as a photographer made his lectures particularly interesting and added substantially to the impact of his publications as well. In this context, his extensive collaboration with his wife, Maria, who became an expert in electron microscopy, is especially noteworthy. Many of his papers in *Science* appeared in issues whose cover pictures he provided.

No account of Tom Eisner’s life should omit mention of his love of music. He was an outstanding pianist and had the remarkable ability to sight-read just about anything placed before him as if it were something he had been playing for years. He especially enjoyed inviting old and new friends and visitors to his home for musical evenings, which might be spent reading through anything from baroque flute sonatas and Beethoven chamber works to Schubert Lieder, Bach arias, or even complete Mozart operas. He also enjoyed conducting and took great pleasure in leading an amateur orchestra composed of friends and colleagues from the Cornell community. Interestingly, although his love of music was a very important part of his life, he was reluctant to play for an audience. It was the act of making music that gave him the deepest pleasure.

Beyond biology, photography, and music, Tom was engaged passionately in many other activities. He played a very active role in promoting conservation and in defending human rights worldwide. Among his services in these endeavors, he was on the Board of Directors of the National Audubon Society, on the National Scientific Council of the Nature Conservancy, on the World Resources Institute Council, and served as president of the American Society of Naturalists.

Tom Eisner was an exemplary son, a devoted husband, and a proud parent and grandparent. Everything that he undertook, he pursued with intensity and style. His autobiographical volume, *For the Love of Insects*, provides an intriguing account of his remarkable career. He spent his entire independent academic life at one university, never chaired a department, never started a company, and almost never boarded an airplane. He had much to teach us.

SPORE* SERIES WINNER

Science Buddies: Advancing Informal Science Education

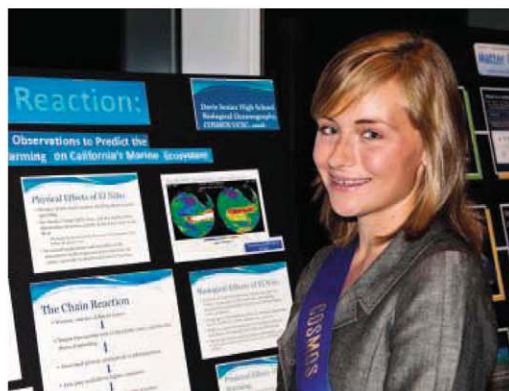
Kenneth L. Hess,† Courtney J. Corda, Kristina M. Lanese

An innovative nonprofit enables scientists to present inspiring project ideas based on their own research to K–12 students, parents, and teachers.

Every year, about 10 million North American kindergarten through high school (K–12) students complete a science project. What part of these science projects is the most difficult? Formulating a hypothesis? Designing an experiment? Analyzing data? The answer: None of the above. Science Buddies (www.sciencebuddies.org)—a nonprofit developer of software, content, and other resources for informal science and engineering education—found that students have the most difficulty selecting a topic and doing background research to inform their work. Meanwhile, scientists and engineers interested in advising and inspiring students often do not have a means to connect with them (see the first figure). To Science Buddies, these difficulties presented an ideal challenge, and we applied a business methodology to address them, viewing our users as customers and our output as products.

After analyzing customer needs, we created free products that include personalized learning tools, 15,000 pages of scientist-developed subject matter, including experiments based on the latest research, and an online community of science professionals willing to give advice to students. We also provide resources to support parents and teachers as they guide students doing hands-on science projects. Representing much more than a Web site, Science Buddies quickly and efficiently matches students with information that will hold their interest and answer their questions. The result is that, during 2010, 9.8 million unique individuals visited the Science Buddies Web site (1), a number equal to ~18% of U.S. students in grades K–12.

One of our programs, the Academic Outreach Partnership program provides an effective method for scientists and engineers to communicate their research to students. Through this program, a scientist can publish a “project idea” outlining how a student can conduct an experiment based on cutting-



Science fair contestant. Science Buddies provides a bridge between scientists and students, enabling students to do projects based on current scientific research.

edge scientific research on the Science Buddies Web site, which shows students how science works and what scientists do every day. For example, Martin Wikelski and his team at Princeton developed “Are we there yet? Test how migratory birds navigate” (2). This project idea uses the team’s radio telemetry data on the orientation of migratory songbirds to enable students to conduct their own version of the study (3). Similarly, Elizabeth Young’s team at MIT developed “From water to fuel to water: The fuel cycle of the future,” which allows students to explore new catalysts for splitting water, an exciting area of active research in renewable energy technology (4, 5). The Science Buddies Web site is a highly effective marketplace for such outreach, aggregating across time and place large numbers of students self-identified as interested in a field and connecting them with researchers in that discipline. We welcome researchers to contact us about participating in this publishing program. On average, about 14,000 unique individuals access each project idea every year.

Our visitor demographics closely reflect the diversity of the U.S. student population; consequently, our library of project ideas must address widely varying student ages, abilities, resources, and need for instructional supports or scaffolding (6). A typical project idea includes a brief introduction, expected duration and cost, safety issues, prerequisites, a list of important terms, a materials

and equipment list, and an experimental procedure that varies in completeness depending on the grade level of the student and the nature of the experiment. For students who need less direction, each project idea suggests a number of variations without providing the detail of a full project idea. We also have hundreds of abbreviated project ideas that provide no scaffold at all. In aggregate, students gravitate to projects with a level of difficulty consistent with their situation; they do not just pick the easy ones!

Science Buddies’ staff scientists have supplemented projects submitted as part of our Academic Outreach Partnerships with a library of more than 1000 project ideas covering topics in 30 scientific fields. As part of our focus on customer needs, we monitor which areas of science receive the most interest, develop new project ideas in the areas with the most traffic, and always try to broaden the scope of scientific concepts covered. We encourage student feedback that enables us to continuously update and improve existing project ideas.

Because students have difficulty finding a topic to work on and because we strongly believe that students learn the most from a project when they have an intrinsic interest in the subject matter, we developed an “intelligent” recommender for the Science Buddies Web site (see the chart). The Topic Selection Wizard asks questions about everyday interests and grade level and then directs students to matching project ideas from our library. In 2008, the National Academy of Engineering identified the advancement of personalized learning and, specifically, recommender systems for education, as 1 of 14 grand challenges in engineering for the 21st century (7). The performance of our Topic Selection Wizard substantiates the value of such tools. Students who use the Wizard are 95% more likely to find a project idea that satisfies them compared with students who simply browse possible projects.

Putting a face on the numbers, one parent told us that “[his fourth-grade daughter] is into stuffed animals, loves pets, and loves the Sierra Nevada camp with its bugs, birds

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*SPORE, Science Prize for Online Resources in Education; www.sciencemag.org/site/special/spore/.

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& snakes, where my wife is director. I completely expected her to do an animal project and was prepared to talk about ethics and animal-use protocols. Well, as she went through the [Wizard] questions, what we discovered is that she's deeply interested in electricity, devices, and math-oriented topics. This was a real gift to all of us." This student completed a project on static electricity, won an award at her school science fair, and reported to us, "Science is fun!"

Having a topic for a project is just the beginning. Our Web site has project guides that provide instruction on every step of the scientific method and the engineering design process. It also offers materials and guidance to develop strong researching and writing skills, which are essential to scientific practices and discourse.

For students excited about their work, project ideas link to extensive, student-friendly career profiles for related science and engineering areas. We also believe it is important for students to interact directly

with scientists. Each year, science and engineering professionals volunteer their time to answer questions from students on our Ask an Expert online bulletin board. To insure a safe learning environment, all adult volunteers must pass annual background checks, and all communications with students occur in a public, moderated forum. Although most students obtain the information they need with just one or two posts, on occasion a true mentoring relationship ensues. One example is the nearly 2-year-long tutelage of high school student Christina Wang by Science Buddies Experts, especially Donna Hardy, a dedicated volunteer from Bio-Rad Laboratories. In a correspondence that comprised more than 100 posts, Miss Wang got inspiration, ideas, and practical guidance that led her from knowing virtually nothing about biotechnology to developing a screen for detecting biofilm-inhibiting metabolites in four bacteria species. Her project won a second-place Grand Award at the 2010 Intel International Science and Engineering Fair.

By providing tools that empower students and offering guides and best practices for teachers, Science Buddies makes it easy for teachers to incorporate student-led science investigations in their curriculum. Deb Bogard, a veteran middle school science teacher, says that she has "been using Science Buddies materials for 6 years, and the quality of my students' projects and learning has increased exponentially. Parents confided in me that at first, they and their children felt intimidated by the amount of work that a science project required. However, as the students began working, they found that the way the project was broken down into manageable pieces, and the instruction that was provided by the Science Buddies project guide, enabled them to take charge of their own learning. They now viewed the science project as one of the most valuable learning experiences of their school career."

The Web site is popular and widely recommended because it is free, serious, constantly maintained, and

About the authors



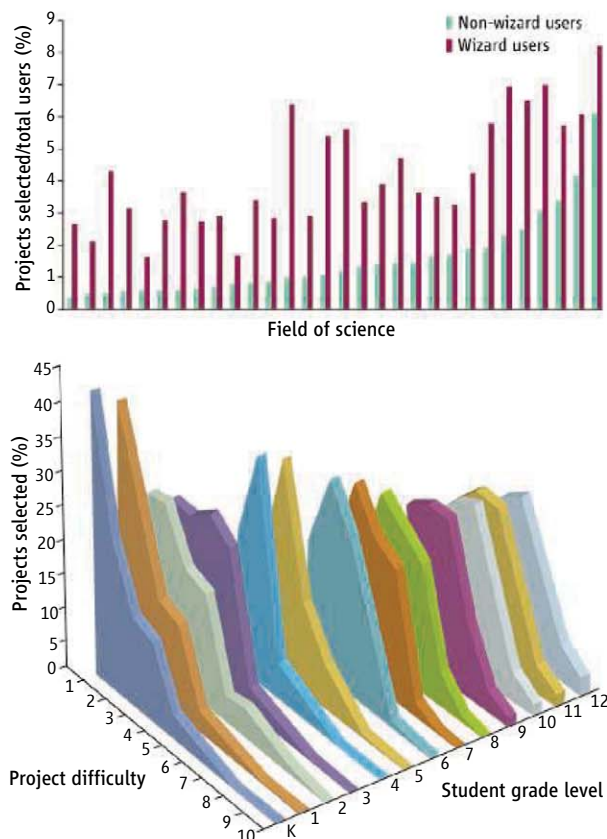
Courtney Corda is vice president of Science Buddies. **Ken Hess** is founder and president of Science Buddies and chairman of the California State Summer School for Mathematics and Science (COSMOS) Advisory Board. **Tina Lanese** is vice president of Science Buddies.

effectively marketed. We are working hard to keep it that way by developing a diverse base of funding from corporations, private foundations, and individuals.

By targeting a known need for science resources among K–12 students doing science projects and by addressing the specific problems of those customers with personalized learning tools, rich content, connections to practicing scientists, and forums for discussion, Science Buddies has built a thriving community of learning. We believe that there are many other occasions, often outside a formal educational setting, where an untrained citizen has a strong need to learn science and engineering concepts. We intend to apply a similar approach to these situations to inspire students to study science and increase the science literacy of society.

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6. Slightly more than half (55%) of the 2010 student visitors are female. Student visitors represented ethnicities of Caucasian (32%); Hispanic (12%); African-American (11%); and Alaska Native, Asian, Asian Indian, Native American, or Pacific Islander (less than 5% each), with 27% declining to state their ethnicity.
7. National Academy of Engineering, Advance personalized learning; www.engineeringchallenges.org/cms/8996/9127.aspx.



Topic Selection Wizard effects. An "intelligent" recommender, the Topic Selection Wizard, helps students discover their true interests. **(Top)** Those who do not use the Wizard tend to focus on projects in fields they have heard of, so fields like "food science" are overrepresented. Wizard users are 95% more likely to find a project they like and tend to be less influenced by how well known the field is. **(Bottom)** Students naturally gravitate to projects with a difficulty appropriate to their grade level.



EDUCATION

AAAS Testing Web Site Probes Students' Misconceptions About Science

First, the good news: 95% of middle and high school students know that DNA contains genetic information that is passed from parent to offspring. But 57% of them incorrectly believe that a gene is a trait—like having blue eyes—and not a segment of DNA.

Those insights and others like them are at the core of a new assessment Web site developed by AAAS Project 2061, the science literacy initiative, to track what students know—and don't know—about science. With a focus on students' misconceptions, the site is a practical guide for educators as they create multiple-choice tests and diagnose whether their students truly understand the science concepts they are taught.

With increasing calls for national science standards and a common core curriculum for middle school and high school students, it is more important than ever to get assessment right, said George DeBoer, deputy director of Project 2061.

"Good assessments can be used to actually improve students' learning and not just to hold teachers and schools accountable," said DeBoer, the principal investigator for the project. "Assessments that are designed to diagnose students' misconceptions can be powerful educational tools."

AAAS's Project 2061, founded in 1985 as a long-term effort to improve science education, began work on the assessment program in 2004 with support from the National Science Foundation. Its Web site (<http://assessment.aaas.org>) presents detailed information on how a national sample of students answered 600 multiple-choice questions on topics from cell biology to plate tectonics. The test questions were developed by AAAS staff and doctorate-level research associates, and evaluated by experts in science, science education, and science assessment.

Each question typically was answered by at least 2000 students in field tests involving

school districts across the nation. In 2010, for example, more than 90,000 students in 814 schools participated in the field tests. Project 2061 researchers also conducted on-site interviews with students to gauge the effectiveness of the questions.

As a whole, the new Web site offers an unusually detailed picture of what middle and early high school students across the United States know about science, broken out by grade level, gender, and primary language.

There was "essentially zero" difference in how well girls and boys answered the questions, said DeBoer, with only two-

The assessments also counter the widely held view that multiple-choice questions are useful only for testing recall of memorized definitions and trivial facts, DeBoer said. "As a result of our efforts, many of the test questions included in the new Web site not only measure knowledge of factual information, but they also probe a student's ability to explain real-world phenomena, reason logically through problem situations, or identify the reason why a claim is true," he explained.

The site has received early positive reviews from teachers and curriculum specialists, who noted that the AAAS assessment contains valuable information beyond the data typically contained in progress reports such as the National Assessment of Educational Progress or the Trends in International Mathematics and Science Study.

The site's emphasis on student misconceptions is particularly helpful, said Deagan Andrews, a curriculum and assessment specialist in the Greeley, Colorado, school district. Referring to some of the major standardized test programs, he said: "No one releases any information about misconceptions. They are interested in whether students got it right or wrong."

AAAS's assessment, Andrews added, offers teachers an opportunity to "think about their instruction and what they may be doing that may be perpetuating misconceptions."

"It becomes more difficult to teach students without actually addressing the misconception first," agreed Anu Malipatil, a school administrator for a network of charter schools in New York and Connecticut. The AAAS test items, she said, can be used by teachers to diagnose potential problem areas and find out how many students in a classroom have misconceptions about key concepts.

—Earl Lane

Which statement about matter is TRUE?

- (A) Atoms are not matter but they are contained in matter.
- (B) Matter exists only when you can see it.
- (C) Living things are not matter.
- (D) Matter is made up of atoms.

The answer is D. Nearly 70% of students tested by Project 2061 answered correctly, but 17% chose answer A. By offering insight on students' misconceptions, the new assessments Web site can help shape more effective teaching.

tenths of a percentage point separating their scores. Students whose primary language was not English scored seven percentage points lower on the test, even though the AAAS team took pains to address issues of potential bias and used plain language in the questions.

Alzheimer's Experts Share Diverse Insights on Disease

Some 5.4 million Americans have Alzheimer's disease, and given longer lifespans ahead, "we're facing quite a crisis," David M. Holtzman said during a recent AAAS event intended to stimulate new thinking and speed medical advances. The number of Alzheimer's patients is likely to triple within 50 years, but researchers still can't detect the disease until it's too late to prevent dementia and death, said Holtzman of the Washington University School of Medicine in St. Louis (WUSTL).

Yet, optimism prevailed 6 April as researchers spoke to a packed AAAS auditorium about "Breaching Barriers in Alzheimer's Disease." Reisa Sperling of Brigham and Women's Hospital and Massachusetts General Hospital announced a proposal for a new clinical trial. Other speakers described insights into amyloid plaque formation in the brain—a hallmark of the disease—as well as genetic factors, imaging techniques, and lessons from other neurodegenerative diseases.

Late-onset Alzheimer's disease, affecting people who are 60 or older, accounts for 99% of all cases. Although much rarer, the early-onset familial form can be especially devastating, striking those as young as 30. "Age and family history are the biggest risk factors," explained WUSTL's Alison Goate. "Even though genes are playing a role, that's not all it is," she said. A number of disease-causing genetic mutations as well as the risk factor apolipoprotein E4 have been identified.

Remaining physically active can promote overall good health, Sperling said, but avoiding Alzheimer's isn't as simple as playing Sudoku. As Mark Mintun of Avid Radiopharmaceuticals noted, a gene-environment interaction seems likely. Two radiotracers, combined with PET scanning, make it possible to visualize amyloid plaques, he said. Cerebrospinal fluid analysis can also be used to detect the plaque components tau and amyloid-beta 42 peptide, but Holtzman said high levels probably signal advanced deterioration, even if cognitive symptoms are mild.

An array of presentations offered support for the amyloid hypothesis, which assumes that amyloid-beta peptide is the key culprit in plaque formation. But Mathias Jucker of the Hertie Institute for Clinical Brain Research and the University of Tübingen also posed unconventional questions, such as whether environmental factors may trigger plaque formation. Steven Finkbeiner of the Gladstone Institute of Neurological Disease asked whether neuroinflammation may be a common thread among various neurodegenera-

tive diseases. Don W. Cleveland of the University of California, San Diego urged looking beyond neurons to assess the role of other brain cells.

Richard Ransohoff of the Cleveland Clinic offered intriguing clues to the function of nonneural cells called microglia and



Graceful Aging.

France's Jeanne Louise Calment lived to be 122 years old, riding a bicycle at age 100. Andrew Dillin of the Salk Institute wants to know how she beat the genetic odds.

a microglia modulator, the fractalkine receptor, which seems to affect disease outcomes. Andrew Dillin of the Salk Institute for Biological Studies described an aging "toggle switch" that might help prevent diseases that involve protein misfolding. Sperling proposed a trial to "test the hypothesis that interfering with amyloid accumulation upstream will affect downstream neurodegeneration."

The public event, supported by The Agouron Institute, honored the late *Science* editor emeritus and AAAS senior advisor Philip Hauge Abelson. A related workshop, chaired by Marc Tessier-Lavigne of The Rockefeller University and Tom Maniatis of Columbia University Medical Center, will result in a white paper in *Science Translational Medicine*.

(For video and a complete list of speakers, see www.aaas.org/go/abelson2011.)

—Ginger Pinholster

INTERNATIONAL

Asia-Pacific Leaders Explore Ambitious S&T Cooperation

A group of leaders from the Asia-Pacific science community, convened by AAAS, explored a selection of possible actions that could be taken by governments, universities, funding agencies, and businesses to strengthen cooperation and innovation throughout the region.

In all, the roundtable involving more than 30 leaders from across the region yielded nearly two dozen possible steps for developing "a more coherent and compatible scientific system." According to a summary of the event released this month, ideas ranged from identifying common challenges and developing shared ethics to making universities hubs for regional collaboration and problem-solving.

The discussion showed how increased science cooperation could play a vital role in supporting the Asia-Pacific research enterprise, said AAAS Board Chair Alice S. Huang: "Given the growth and potential contributions from scientific developments in the Pacific Rim countries, the extraordinarily candid and focused discussion during this initial meeting was an extremely promising start to productive collaboration among scientists and institutions in this part of the world."

The roundtable, organized by Huang and the AAAS International Office, brought scientists, policy-makers, educators, and others to AAAS headquarters for a full day of discussion on 16 February, the eve of the association's Annual Meeting. They met under Chatham House Rule, which encourages a frank exchange of ideas by

assuring that participants will not be identified or quoted.

The Asia-Pacific region—which includes nations as diverse as China, India, Japan, Australia, Mexico, and Canada—accounts for more than 40% of the world's population and over half of its economic output. It is also a growing science power. But while important networks such as the Asia-Pacific Economic Cooperation forum have developed, the summary suggests that more support is needed from political leaders to drive science cooperation.

As a start, it says, they "could endorse informal, regular discussions by scientific leaders (e.g., science advisers) on S&T cooperation to address regional challenges, barriers, and opportunities."

Among other potential steps outlined in the five-page summary:

- Broad efforts to build partnerships among universities, including curriculum, faculty exchanges, and tenure policies to support regional science;
- Collaboration by policy-makers, educators, funding agencies, and others to identify regional issues—and mechanisms for addressing them;
- Initiatives led by science organizations to build compatible scientific norms and ethics that could help knit together a regional scientific culture.

(Read the full summary at www.aaas.org/go/191summary.)



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- Effects of social media, Web 2.0, and patient-driven networks on clinical research
- Clinical research's response to public health needs
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- Forging government-industry partnerships
- Using clinical trials to improve patient care

The keynote address will be delivered by
Robert Califf, MD, MACC

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Microresonator-Based Optical Frequency Combs

T. J. Kippenberg,^{1,3*} R. Holzwarth,^{2,3} S. A. Diddams^{4*}

The series of precisely spaced, sharp spectral lines that form an optical frequency comb is enabling unprecedented measurement capabilities and new applications in a wide range of topics that include precision spectroscopy, atomic clocks, ultracold gases, and molecular fingerprinting. A new optical frequency comb generation principle has emerged that uses parametric frequency conversion in high resonance quality factor (Q) microresonators. This approach provides access to high repetition rates in the range of 10 to 1000 gigahertz through compact, chip-scale integration, permitting an increased number of comb applications, such as in astronomy, microwave photonics, or telecommunications. We review this emerging area and discuss opportunities that it presents for novel technologies as well as for fundamental science.

The optical frequency comb (1, 2) provides the “light gear” that enables a reliable and accurate means of counting optical cycles on the femtosecond (10^{-15} s) time scale, as required for the realization of optical clocks (3). The accuracy of such clocks is now measured to the 18th digit, exceeding the performance of their microwave counterparts by more than an order of magnitude (4). This development involves the measurement of the energy structure of atoms at an unprecedented level, allowing some of the most precise laboratory tests of the physics governing these systems (5, 6). Beyond their use in optical clocks and precision spectroscopy, frequency combs have found widespread use in various emerging research areas, including attosecond pulse generation, ultraviolet and infrared (IR) spectroscopy (7–9), precision distance measurement (10), remote sensing, optical waveform (11) and microwave signal synthesis (12), and astronomical spectrograph calibration (13, 14). These applications all benefit from the broad spectral coverage of the precisely controlled frequency components that constitute the optical comb.

To date, most of these applications have been realized with optical frequency combs based on femtosecond lasers—principally Ti:sapphire, Er:fiber, and Yb:fiber mode-locked lasers. Although fiber lasers have enabled optical frequency combs to evolve into a robust turnkey and commercial technology, for many interesting applications it would be advantageous to further reduce the footprint and at the same time to increase the repetition rate into the frequency range above 10 GHz. In recent years, a new comb generation principle has emerged that uses parametric frequency

conversion in compact optical microresonators (15). This approach may provide a new generation of combs that enable planar integration and may permit a direct link from the radio frequency (RF) to optical domain on a chip.

Principles of Frequency Combs

It is instructive to briefly review the fundamental principles of optical frequency combs based on mode-locked lasers (1, 16). The frequency domain spectrum of the output of a mode-locked laser constitutes an optical frequency comb. The comb arises from the periodic train of pulses emitted

from such a laser (Fig. 1A). The spacing of the comb modes is given by the repetition rate (f_r) of the laser, which is the inverse of the time required for an optical pulse to circulate through the laser cavity. Successive pulses emitted from a mode-locked laser are not generally identical. The difference between phase and group velocity in dispersive media inside the laser cavity causes the pulse envelope to slip with respect to the carrier phase from pulse to pulse (Fig. 1A inset), leading to a global offset of the frequency comb (f_o). Correspondingly, the frequencies of the optical modes are then given by $f_n = f_o + n \times f_r$, where n is a large integer mode index (e.g., $n \sim 10^6$ for $f_r = 100$ MHz and an optical frequency $f_n \sim 10^{14}$ Hz). Measurement and frequency control of f_r and f_o (17–19) therefore provide a direct phase-coherent link between optical and RF domains, which is the basis of many frequency comb applications.

Frequency Comb Generation Using Microresonators

Combs can also be produced from a continuous wave (CW) laser through the nonlinear optical process of parametric frequency conversion (Fig. 1, B and C) in optical microresonators (15) that trap and confine light in small volumes and thereby enhance the light intensity and nonlinear interaction (20). An important class of microresonators are whispering gallery mode (WGM) resonators—such as microdisks, microspheres, microtoroids, or microrings—which confine light by total internal reflection around the perimeter of an

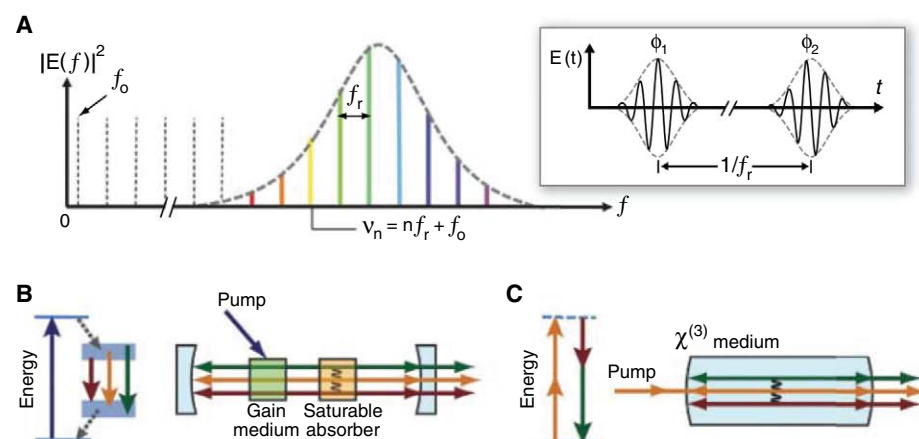


Fig. 1. (A) Time and frequency domain representation of an optical frequency comb. The mode spacing is given by the pulse repetition rate (f_r), and the offset frequency (f_o) describes the evolution of the carrier-envelope phase $\Delta\phi = \phi_2 - \phi_1$, which is the difference in phase between the pulse envelope and carrier between successive pulses (see inset). This phase difference ($\Delta\phi$) leads to a global offset of the frequency comb given by $f_o = f_r \Delta\phi/(2\pi)$. (B) Conventional frequency comb generators based on mode-locked lasers, which—due to their pulsed operation—emit a comb of optical frequencies. Mode-locked lasers typically contain a gain medium (externally pumped, green arrow), a medium that induces mode locking (such as a saturable absorber), and a laser cavity. (C) Schematic of the parametric optical frequency comb generation method (15). The pump laser is part of the optical comb and injected into a resonator. Although a linear resonator cavity is shown here, in many situations, the resonator comprises a whispering-gallery mode microresonator. In contrast to the laser oscillator that has its gain medium pumped into an excited state and emits photons via stimulated emission, in the parametric oscillator the $\chi^{(3)}$ nonlinear material has no energy storage but acts to convert two pump photons into signal and idler photons through virtual energy levels. This process is mediated by the intensity dependent refractive index n_2 (also termed Kerr coefficient).

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air-dielectric interface. It has been first demonstrated in optical microspheres (21) that ultrahigh Q resonances (>100 million) can be attained (where $Q = \omega\tau$, the resonance quality factor, with τ denoting the photon storage time and ω the optical angular frequency). This ultrahigh Q factor results in long interaction lengths and can lead to extremely low thresholds for nonlinear optical effects (sub micro-Watt power level). The optical WGM resonances correspond to an integer number of optical wavelengths (the mode index n) around the microresonator's perimeter and are separated by the free spectral range (FSR), that is, the inverse round trip time in the cavity (Fig. 2C).

In the case of resonators made of fused silica, silicon, or crystals that exhibit inversion symmetry, the elemental nonlinear interaction is third-order in the electric field, which gives rise to the process of parametric four-wave mixing (FWM). This frequency conversion process originates from the intensity-dependent refractive index, $n_0 + I \times n_2$, where n_2 is the Kerr coefficient, n_0 is the linear refractive index, and I denotes the laser intensity. When a microresonator made from a third-order nonlinearity material is pumped with a CW laser (Figs. 1C and 2A), this parametric frequency conversion will annihilate two pump photons (with angular frequency ω_p) and create a new pair of photons: a frequency upshifted signal (ω_s), and a frequency downshifted idler (ω_i). The conservation of energy ($2\hbar\omega_p = \hbar\omega_i + \hbar\omega_s$, where $\hbar$ is the reduced Planck constant) implies that the frequency components are equally spaced with respect to the pump (i.e., $\omega_s = \omega_p + \Omega$ and $\omega_i = \omega_p - \Omega$, where 2Ω is the frequency separation of the two new sidebands). If the signal and idler frequencies coincide with optical microresonator modes (Fig. 2C), the parametric process is enhanced, resulting in efficient sideband generation. Momentum conservation is satisfied in this process, since the WGMs have a propagation constant $\beta = n/R$ (where n is the mode index and R the resonator radius) such that $2\beta_p = \beta_s + \beta_i$ for symmetrically spaced modes [i.e., signal and idler modes differing by an equal amount (Fig. 2C)].

The generated sidebands have a defined phase relationship, that is, the relative phases of signal and idler with respect to the pump are fixed. If the scattering rate into the signal and idler modes exceeds their respective optical cavity decay rates (κ) (Fig. 2C), parametric oscillation occurs, leading to symmetric sidebands that grow in intensity with increasing pump power. Although these effects are well known in nonlinear optics, such a process was demonstrated only recently in silica (22) and crystalline (23) microresonators. The advantage of microresonators is that the threshold for initiation of parametric oscillation can be strongly reduced, because the threshold scales with the inverse Q factor squared, implying that high Q can give a dramatic reduction in required optical power.

Parametric oscillations can also lead to spectra that contain multiple sidebands. The spectral band-

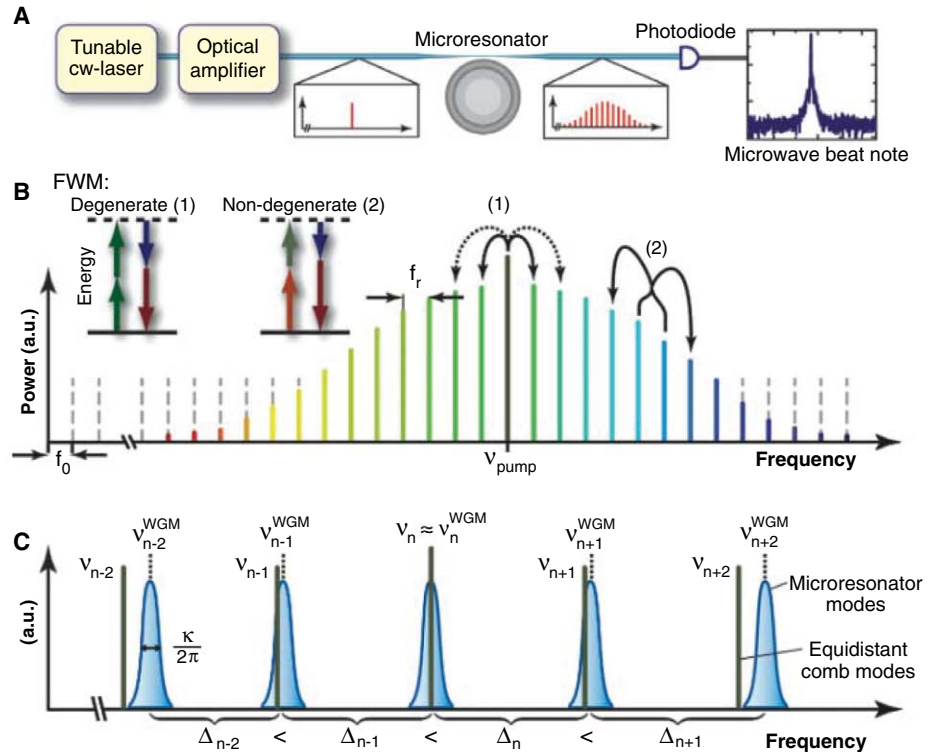


Fig. 2. Principle of optical frequency comb generation using optical microresonators. **(A)** An optical microresonator (here, a silica toroid microresonator) is pumped with a CW laser beam. The high intensity in the resonators ($\sim \text{GW}/\text{cm}^2$) gives rise to a parametric frequency conversion through both degenerate and nondegenerate (i.e., cascaded) FWM. Upon generation of an optical frequency comb, the resulting beatnote (given by the inverse cavity round-trip time) can be recorded on a photodiode and used for further stabilization or directly in applications. **(B)** Optical frequency comb spectrum, which is characterized by the repetition rate (f_r) and the carrier envelope frequency (f_0). In the case of a microresonator-based frequency comb, the pump laser is part of the optical comb. The comb is generated by a combination of degenerate FWM (process 1, which converts two photons of the same frequency into a frequency upshifted and downshifted pair of photons) and nondegenerate FWM (process 2, in which all four photons have different frequencies). The dotted lines indicate degenerate FWM into resonator modes that differ by more than one mode number. The presence of cascaded FWM is the underlying process that couples the phases of all modes in the comb and allows transfer of the equidistant mode spacing across the entire comb. **(C)** Schematic of the microresonator modes (blue) and the frequency comb components (green) generated by pumping a whispering-gallery mode with a pump laser. The mode index (n) refers to the number of wavelengths around the microresonator's perimeter. The FWM process results in equidistant sidebands. The bandwidth of the comb is limited by the variation of the resonator's free spectral range (Δ_n) with wavelength due to dispersion (shown is the case of anomalous dispersion).

width can be increased by two nonlinear processes (Fig. 2B). First, the pump laser can convert pump photons to secondary sidebands, spaced by multiple free-spectral ranges of the cavity (Fig. 2B). This degenerate FWM process would again lead to pairwise equidistant sidebands. However, the generated pairs of sidebands are not necessarily mutually equidistant, that is, they are not required to have the same frequency separation, as is needed to form a comb.

On the contrary, in a second process, comb generation can occur when the generated signal and idler sidebands themselves serve as seeds for further parametric frequency conversion, which is also referred to as cascaded FWM (also termed nondegenerate FWM because the two pump photons have different frequencies) (Fig. 2B). When signal and idler sidebands have comparable power

levels to that of the pump inside the cavity, cascaded FWM is the dominant process by which new sidebands are generated. This process leads to the generation of equidistant sidebands, that is, all generated frequency components have the same separation from each other, giving rise to an optical frequency comb.

Dispersion, the variation of the free spectral range of the cavity with wavelength, ultimately limits this conversion process and leads to a finite bandwidth of the comb generation process because the cascaded FWM is less efficient once the comb modes are not commensurate with the cavity mode spectrum (Fig. 2C). Interestingly, however, the bandwidth of the comb is not entirely determined by the dispersion of the microresonator alone. Indeed, the nonlinear optical mode pulling (22) that occurs due to the Kerr nonlinearity at

high power plays an important role and thereby extends the comb beyond the limits imposed by dispersion alone (24).

An important aspect of the optical frequency comb in metrology is the stabilization of the comb repetition rate and carrier envelope frequency. Although microresonators do not feature any moveable parts, high-speed control of both f_o and f_r can be attained in an independent manner (25). In particular, f_o is directly accessible by varying the pump laser frequency with respect to the cavity mode. In contrast, the repetition rate can be varied by use of the intensity-dependent round-trip time of the cavity. Due to both thermal effects (i.e., the change of refractive index due to heating by absorbed laser power) and the Kerr nonlinearity, power variations of the laser are converted to variations in the effective path length of the microresonator and therefore change the mode spacing of the frequency comb (25). By using electronic feedback on both laser frequency and laser power, a microresonator Kerr comb with a mode spacing of 88 GHz has been fully frequency stabilized, with an Er:fiber laser-based frequency comb serving as reference.

Experimental Systems: From Microtoroids to Integrated Chip-Scale Combs

The requirements for optical comb generation with a microresonator are a high Q cavity with small mode volume that is made from a material with a third-order nonlinearity and low dispersion. Examples of different resonators are shown in Fig. 3D. Toroidal microresonators (26) were the first system in which optical frequency comb generation was demonstrated (15). They consist of a microscale silica toroidal WGM and can attain Q factors in excess of 100 million (where Q is the product of optical angular frequency and photon lifetime). Highly efficient coupling into these planar devices can be achieved by using the evanescent field of tapered optical fibers (i.e., fibers whose diameter is less than, or on the order of, an optical wavelength in diameter, as illustrated in Fig. 2A). Overcoupling, the regime where the total cavity losses are dominated by useful output coupling “loss” into the fiber waveguide, has been achieved, which is important to attain high conversion efficiency, as only outcoupled comb components are detected. Using tapered fiber coupling of ~100 mW pump power at 1550 nm, a 375-GHz repetition rate frequency comb was attained with a spectral bandwidth exceeding 350 nm (15). This broad bandwidth was achieved with intrinsic dispersion compensation. A typical WGM microresonator features normal dispersion (i.e., high optical frequencies propagate slower than low optical frequencies), because a high-frequency mode will have its field maximum closer to the cavity boundary than a low frequency mode. This can be compensated with the material dispersion of silica that exhibits opposite behavior leading to a zero dispersion wavelength in the 1550-nm region. Pumping around this wavelength leads to the creation of broad

frequency combs that can exhibit more than a full octave (a factor of two in frequency), i.e., a span from 1000 to 2200 nm in wavelength as shown in Fig. 3A (24). That the emission indeed constitutes an optical frequency comb has been verified experimentally using multiheterodyne spectroscopy (9). The uniformity of the mode spacing was shown to be better than 1 part in 10^{17} (15). These developments in microresonator-based combs have therefore closely followed the early work in femtosecond laser-based frequency combs, which was equally focused to verify the comb modes’ equidistant mode spacing at a similar level of precision (1).

A different class of resonators amenable to optical frequency comb generation are crystalline resonators (23). These are millimeter-scale resonators, made by polishing a cylindrical blank, that feature exceptional Q factors that exceed 10^{10} and lead to an optical finesse $>10^7$. Input and output coupling of light is achieved with evanescent prismatic couplers, and optical frequency combs with a mode spacing as low as 12 GHz have been attained (27). This frequency is low enough to be directly detected using a photodetector, and such microresonators have been employed to generate microwave signals having high spectral purity (25, 28).

In addition to whispering gallery microresonators, Fabry-Perot fiber-based cavities can equal-

ly give rise to optical comb generation (29) by using an interplay of the third-order nonlinearity and Brillouin scattering. The Brillouin effect is due to the scattering of a photon by an acoustic phonon in the glass fiber and normally leads to a reflected field with a lower frequency (shifted by the phonon frequency of ~10 GHz in the case of silica). Because the acoustic phonons in most conventional materials (such as glass) are rapidly damped, Brillouin scattering is normally a phase-insensitive process. However, in the case of strongly cascaded Brillouin scattering (and in the simultaneous presence of FWM), even this process can lead to the emission of a comb of phase-locked frequency components.

Optical frequency combs have also been generated in more compact silicon photonic circuits that integrate both resonator and waveguide on the same chip. An important advance in this direction has been the recent demonstration of optical frequency comb generation in integrated silicon nitride (SiN) resonators (30) fabricated using approaches compatible with widespread complementary metal-oxide semiconductor (CMOS) technology. Although the optical Q factor remains many orders of magnitude lower ($Q=10^5$ to 10^6) than in the case of silica or crystalline resonators, this is partially compensated for by the tight confinement of the field inside the microresonators

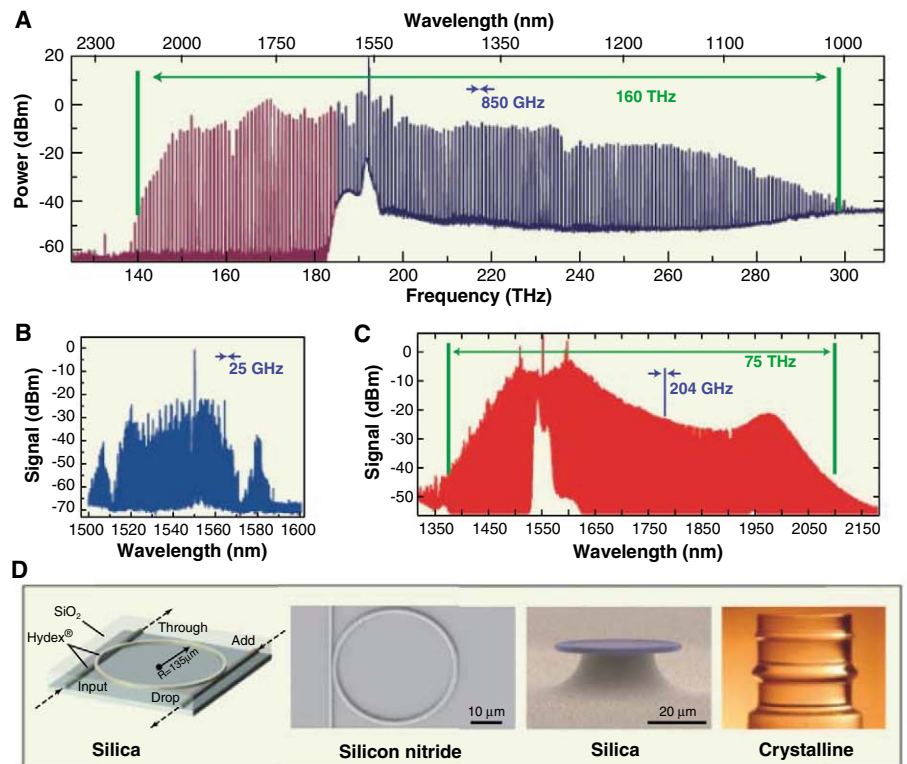


Fig. 3. Microresonator-based frequency combs. (A) Spectrum of an octave-spanning frequency comb generated using a silica microtoroidal resonator (24). (B) An optical frequency comb generated using a crystalline CaF_2 resonator with a mode spacing of 25 GHz (27). (C) Optical spectrum covering two-thirds of an octave (with a mode spacing of 204 GHz) generated using an integrated SiN resonator (31). (D) Experimental systems in which frequency combs have been generated (from left to right): Silica waveguides on a chip (Hydex glass) (32), chip-based silicon nitride (SiN) ring resonators (30) and waveguides, ultrahigh Q toroidal microresonators (24) on a silicon chip, and ultrahigh Q millimeter-scale crystalline resonators (27).

(Fig. 3) and the fact that SiN exhibits a third-order nonlinearity of $n_2 = 2.5 \times 10^{-15} \text{ cm}^2/\text{Watt}$, which is approximately one order of magnitude larger than that of silica or crystalline materials such as CaF_2 or MgF_2 .

The main benefit of this approach is that it is fully planar and offers considerable flexibility, such as access to dispersion engineering through suitable resonator coatings. Moreover, this approach allows direct integration of the waveguide on the same chip-scale platform providing a means to fabricate a compact packaged device. Using an integrated SiN resonator, optical frequency comb generation has recently been demonstrated in this manner (31). As shown in Fig. 3, two-thirds of an octave with mode spacing of 204 GHz could be attained when pumping at 1550 nm with 300 mW of CW power (31). In addition, parametric comb generation has been demonstrated with another planar integrated resonator using a doped silica glass (Hydex) (32) that is also CMOS compatible and exhibited even higher optical Q factors ($>10^6$).

Planar integration of a frequency comb is a considerable advance (30–32), but it is only part of a much broader class of systems that can be envisioned. In addition to high Q factor cavities and highly efficient coupling techniques, recent advances in silicon nanophotonics (33) have provided on-chip high-speed photodetectors, Raman lasers, modulators, and parametric gain (34). However, FWM near 1550 nm in silicon waveguides suffers from two-photon absorption, which induces optical loss. Thus, more efficient optical frequency comb generation in silicon could be expected in the range >2.2 microns, which is below half the bandgap energy for silicon, suppressing two-photon absorption.

Emerging Applications for Microresonator Combs with High Repetition Rate

Frequency combs with high repetition rates (e.g., 10 to 1000 GHz) are desirable for a number of applications (Fig. 4), but generation is challenging with conventional mode-locked laser-based frequency combs due to the necessity of a short cavity length. In addition, the high repetition rate reduces the peak intensity of a pulse (the enhancement of the peak intensity scales approximately as the total number of comb lines). Nonetheless, repetition rates from ~ 10 GHz to greater than 100 GHz have been achieved in several laser systems (16), notably solid-state mode-locked lasers. The spectral bandwidths of these lasers are typically small ($\Delta\lambda/\lambda < 1\%$), and as a result the peak powers have been insufficient for substantial

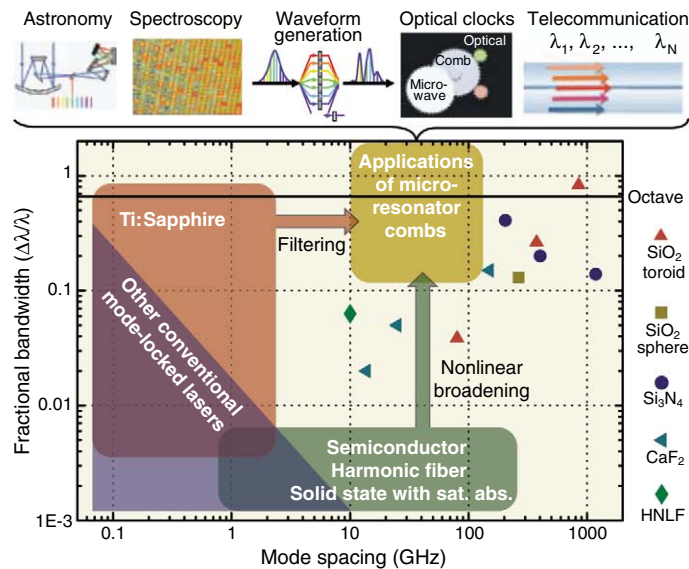


Fig. 4. Optical frequency comb technologies as a function of their mode spacing and fractional bandwidth (where λ denotes wavelength). The bandwidth in this case is defined approximately as the spectral width at which the power in the comb falls below a practically useful power of ~ 1 nW per mode. The high-repetition-rate, large-bandwidth regime (gold colored) is presently approached by a variety of technologies and enables applications in astrophysical spectrometer calibration, telecommunications, optical arbitrary waveform generation, and spectroscopy. The stars denote a sampling of recently demonstrated microresonator-based frequency combs with corresponding material choice of the microresonator. This includes silica toroidal microresonators (15, 24, 25), silica microspheres (40), silicon nitride (31), crystalline CaF_2 resonators (27), and highly nonlinear fiber (HNLF) cavities (29). Applications of microresonator combs are illustrated at the top of the figure.

spectral broadening. One notable exception is a 10-GHz Ti:Sapphire laser that was spectrally broadened to cover an octave, thus enabling full frequency stabilization (35). Mode filtering of a comb with low repetition rate is another option to obtain higher repetition rates but comes at the expense of finite sidemode suppression (because filtering is not perfect), power reduction, and dispersion-induced spectral narrowing. In contrast, microresonator frequency combs naturally provide a high-repetition rate comb, which in some cases can span an octave (24). Although the crystalline resonators (27) have demonstrated mode spacings on the order of a few tens of Gigahertz, those achieved with SiN and silica microresonators are more typically in the range of a few hundred Gigahertz. Increasing the size of the microresonators lowers the repetition rate. However, this also implies that the Q factor of the cavity needs to increase as the radius of the resonator is increased in order to attain the same level of resonant power enhancement (i.e., optical finesse defined as free spectral range divided by the width of the resonance). A particularly interesting set of applications is present for combs with $\Delta\lambda/\lambda > 10\%$ and a repetition rate in the 10 to 100 GHz range, where it is still possible to directly measure the repetition rate with a high-speed photodetector.

Astronomical spectrograph calibration. Precision spectroscopic measurements of periodic Dop-

pler shifts in stellar spectra have led to the discovery of several hundred exoplanets over the past 15 years. Still, the detection of an Earth-like planet within the habitable zone of a distant star has been elusive. This is due in large part because the Doppler shift imparted by such a planet corresponds to a radial velocity of ~ 10 cm/s, nearly an order of magnitude smaller than current detection limits. The ability to measure Doppler shifts near 1 cm/s may possibly allow direct observation of the acceleration of the expansion of the universe (13). Although astronomical spectroscopy with precision approaching 10^{-10} requires numerous advances, it has been proposed that an optical frequency comb could provide a near-ideal calibration grid against which minute Doppler shifts could be measured. Key requirements for such a comb include broad spectral coverage, uniform power distribution, long-term stability, and a comb spacing of three to four times the resolution of the spectrograph. For a typical Echelle spectrograph in the visible or near-IR, the latter condition requires a mode spacing in the range of 10 to 30 GHz. Experiments using a low-repetition-rate laser that is filtered to transmit a sparse comb spectrum

have shown promising results (13, 14). However, a frequency-stabilized optical comb generated with a single laser and a microresonator could be potentially simpler, would be free of unsuppressed side-modes (compared to a filtered low-repetition-rate comb), and could be robustly packaged for unattended long-term operation. Combs with some of these qualities in the near-IR have already been generated in crystalline resonators (27).

Telecommunications. The use of optical frequency combs in the telecommunications bands covering 1450 to 1750 nm has been envisioned, but the channel spacing and data modulation rates pose strict requirements on the mode spacing and power levels. Due to the prevailing use of channels that are spaced by more than 10 GHz with power levels in the range >1 mW, optical frequency combs have not penetrated into this domain. This could change as optical microresonator-based combs reliably generate combs that have high power per comb mode (>1 mW) and additionally can access repetition rates in the range of 25, 50, and 100 GHz, as required for high-capacity telecommunications. The advantage of the optical comb generator is that it can simultaneously generate hundreds of telecommunication channels from a single low-power off-chip source. Thus, a single high-power laser source and a microresonator-based comb can in principle replace the individual lasers used for each channel in telecommunications.

Optical and microwave waveform synthesis.

A broad array of uniformly spaced comb modes with milli-Watt-level power would be valuable for applications in the field of microwave photonics and optical arbitrary waveform generation. The large mode spacing from a microresonator is advantageous for optical and microwave waveform generation (11), where recently developed approaches enable Fourier synthesis of user-designed optical waveforms via the control of amplitude and phase of each individual comb line (11). In the optical domain, this may enable generating complex or ultrashort (single cycle) waveforms, or potentially waveforms tailored for quantum control in atomic or molecular processes. When combined with high-speed photodetection, the agile manipulation of comb modes spaced by tens of Gigahertz also provides microwave and millimeter waveforms that are challenging, if not impossible, to synthesize with conventional techniques. Integration of the microresonator comb with advanced dispersive elements and modulators would provide maximal synthesizer functionality in a compact package. Finally, a free-running or stabilized microresonator comb has already been demonstrated to serve as a source of low-phase noise optical or microwave signals (12, 27).

Frequency comb spectroscopy. The fact that a frequency comb consists of a broad bandwidth array of what are essentially CW oscillators makes it an interesting source for directly probing atoms and molecules. Several spectroscopic approaches have been developed for conventional laser combs (36), and it is anticipated that a microresonator-based comb would bring new features of high mode power, compactness, and on-chip integration to this field. In one approach, the modes are spectrally resolved and their amplitudes reveal absorptive features characteristic of the sample gas (37, 38). An alternative multiheterodyne spectroscopy (9) requires two microresonator-based frequency combs (which may in principle reside on the same chip) having slightly differing repetition rates. One comb serves as a local oscillator, whereas the second comb traverses the sample and acquires the spectroscopic fingerprint. The resulting beatnotes of the two combs produce a radio frequency (RF) comb that contains a direct mapping of the optical spectrum, including any absorption and phase shift imparted by the sample (9, 39). Here, a trade-off exists; the high repetition rate gives fast acquisition times but entails coarse sampling of the absorption profile. Indeed, with mode spacing on the order of 10 GHz or greater, one could easily miss narrow spectral features. One solution to this problem would be to scan the microresonator comb, in which case the spectral resolution is ultimately given by the comb linewidth (38).

Future Outlook: Challenges and Opportunities

Microresonator-based frequency combs are still in their development phase, and challenges must be overcome before their full impact is to equal that of femtosecond laser-based frequency combs.

At the same time, the parametric gain underlying these devices provides new opportunities.

One requirement for the use of microresonator combs in many frequency metrology applications will be a robust phase coherent link between RF and optical domains, as provided in the f - $2f$ stabilization of femtosecond laser combs (17, 18). In practice, this requires a spectrum that spans an octave or an appreciable portion thereof. It is encouraging that octave-spanning spectra have already been generated in microtoroid resonators (24); however, an important outstanding aspect will be the frequency noise in the comb modes. Due to the small volume, many processes (such as thermo-refractive and thermo-elastic noise) are enhanced. Phase stabilization will only be viable when the noise on measured f_o and f_r beatnotes is sufficiently low to be compensated with servo-control techniques. A related issue for the planar waveguide and microtoroid systems discussed above is the reduction of f_r to a readily measured frequency below 100 GHz. The requirements of manageable noise processes, spectral coverage over a considerable portion of an octave, and $f_r < 100$ GHz may represent the most important challenges to be overcome in the widespread use of microresonator frequency combs.

A key advantage of the parametric frequency conversion is that, in contrast to molecular and optical transitions of solid-state media, they exhibit broadband gain. Practically, the gain is only limited by the transparency window of the resonator material and dispersion, of which the latter can be engineered. In this context, it is interesting to consider optical comb generation from different materials. Semiconductors such as InP, Ge, SiN, or Si are transparent in the mid-IR. Hence, microresonator-based mid-IR combs could enable a new generation of optical sensing devices in the important spectroscopic “molecular fingerprinting” regime, with a multichannel generator and spectral analysis tools residing on the same chip. A challenge in this endeavor is the engineering of the optical resonators, such that their dispersion is sufficiently low while simultaneously maintaining a high Q factor. In this respect, crystalline resonators based on CaF_2 (23) appear highly promising. The transparency window of CaF_2 (or MgF_2) ranges from 160 nm to $\sim 8 \mu\text{m}$, making this material highly interesting from the perspective of comb generation in the mid-IR (2500 nm onward) as well as wavelengths below 1000 nm.

Summary

Research and development is under way that may allow not only a dramatic reduction in size but also access to comb generators with a repetition rate from 10s of GHz up to ~ 1 THz. For the technology to unfold, it will be necessary to combine the advances from nano- and microphotonics (33) with those of frequency metrology. Microresonator comb generators are not likely to replace existing commercialized laser-based frequency comb generators but may well be the system of choice that can serve the demand for high repetition rates and wavelength ranges such as the

mid-IR. It is clear that the advances made in this rapidly developing field of optical microresonators may well advance optical frequency combs into new and still unanticipated application areas.

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Topological Phase Transition and Texture Inversion in a Tunable Topological Insulator

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The recently discovered three-dimensional or bulk topological insulators are expected to exhibit exotic quantum phenomena. It is believed that a trivial insulator can be twisted into a topological state by modulating the spin-orbit interaction or the crystal lattice, driving the system through a topological quantum phase transition. By directly measuring the topological quantum numbers and invariants, we report the observation of a phase transition in a tunable spin-orbit system, $\text{BiTl}(\text{S}_{1-\delta}\text{Se}_\delta)_2$, in which the topological state formation is visualized. In the topological state, vortex-like polarization states are observed to exhibit three-dimensional vectorial textures, which collectively feature a chirality transition as the spin momentum-locked electrons on the surface go through the zero carrier density point. Such phase transition and texture inversion can be the physical basis for observing fractional charge ($\pm e/2$) and other fractional topological phenomena.

Topological insulators in three dimensions are nonmagnetic insulators with novel surface states that are a consequence of the nontrivial topology of electronic wave functions in the bulk of the materials (1–5). Their experimental discoveries in Bi-Sb semiconductors, and in Bi_2Se_3 and related materials, have led to the exploration of topological quantum phenomena at modest temperatures and without the requirement of any applied magnetic field (6–16). Current theoretical proposals have suggested such intriguing possibilities as the realization of fractional quasi-particles and the exploitation of the role of spin momentum locking, which leads to quantum phases (such as topological Berry's phases), in transport (17–24).

Strong topological insulators are distinguished from ordinary insulators by a finite topological quantum number or an invariant (ν_0) or, equivalently, an axion angle parameter (θ). The value of ν_0 or θ depends on the parity eigenvalues of the wave functions in time reversal-symmetric materials (1, 2) and can be determined from spin texture details of the spin-orbit surface states that form when the bulk is terminated, as demonstrated by Hsieh *et al.* (6–9). In particular, a $\nu_0 =$

$1 = \theta/\pi$ topology requires the terminated surface to have a Fermi surface that supports a nonzero Berry's phase (an odd as opposed to an even multiple of $\pi = \theta = \text{axion angle}$), which is not realizable in an ordinary spin-orbit or trivial ($\nu_0 = 0 = \theta$) insulator. It is believed that a trivial insulator can be twisted into a $\nu_0 = 1 = \theta/\pi$ topological state by signaling the appearance of unusual spin vortex-like [or skyrmion-like (1)] arrangements via increasing spin-orbit interaction or by modulating the lattice parameters, thereby driving the system through a topological quantum phase transition (1, 2). However, the topological insulators Bi_2X_3 ($\text{X} = \text{Se}, \text{Te}$) cannot be tuned out from a trivial insulator version without a structural phase transition; in the Bi-Sb semiconductors, the topological phase transition is masked by an intervening band [the “H” band (1)] and the lack of gating control necessary for its observation.

We demonstrate the existence of a topological phase transition in $\text{TlBi}(\text{S}_{1-\delta}\text{Se}_\delta)_2$, which, as we show, is a fully tunable topological analog of Bi_2Se_3 . By effectively varying the sulfur/selenium ratio (δ), both the spin-orbit strength and lattice parameters are effectively tuned (25). Because the topological order in $\text{TlBi}(\text{S}_{1-\delta}\text{Se}_\delta)_2$ originates from Bi and Se atoms, and to illustrate an analogy with the known topological insulator Bi_2Se_3 (6, 8, 11), we rewrite the formula as $\text{BiTl}(\text{S}_{1-\delta}\text{Se}_\delta)_2$. Figure 1A presents systematic photoemission measurements of electronic states that lie between a pair of time reversal-invariant points or Kramers' points ($\bar{\Gamma}$ and $\bar{M}$) obtained for a series of compositions of the spin-orbit material $\text{BiTl}(\text{S}_{1-\delta}\text{Se}_\delta)_2$. As the selenium concentration is increased, the low-lying bands, separated by a gap of energy 0.15 eV at $\delta = 0.0$, are observed to approach each other; the gap decreases to less than 0.05 eV at $\delta = 0.4$. Both bands demarcating the gap show three-dimensional

(3D) dispersion where binding energies vary with momentum perpendicular to the surface, k_z [as probed via varying incident photon energy (25)], and roughly correspond to the expected position for the valence and conduction bands. The absence of surface states within the bulk gap suggests that the compound is topologically trivial ($\nu_0 = 0 = \theta$) within a composition range of $\delta = 0.0$ to 0.4. Starting from $\delta = 0.6$, a linearly dispersive band connecting the bulk conduction and valence bands emerges, which threads across the bulk band gap. Incident photon energy modulation studies support the assignment of these Dirac-like bands as originating from the surface (25). Moreover, the band continua in the composition range of $\delta = 0.0$ to $\delta = 0.4$ are degenerate, whereas the Dirac-like bands at $\delta = 0.6$ and beyond are spin-polarized (see Figs. 2, 3, and 4).

The system enters a topologically nontrivial phase upon the occurrence of an electronic phase transition between $\delta = 0.4$ and $\delta = 0.6$ at temperatures below 15 K. Although the system approaches the transition from the conventional or no-surface-state side ($\delta = 0.4$), both energy dispersion and Fermi surface mapping (Fig. 1, A and B, for $\delta = 0.4$) show that at the outer boundary of the bulk conduction band continuum that corresponds to the loci where the Dirac surface states would eventually develop, the spectral weight becomes much more intense; however, the surface remains gapped at $\delta = 0.4$, which suggests that the material is still on the trivial side. Finer control of bulk compositional variation does not allow us to locate a precise value for the transition; this could also be a consequence of an intrinsically broader topological transition. A critical signature of a topological phase transition is that the material turns into an indirect bulk band gap material, as conjectured previously (1). As δ varies from 0.0 to 1.0 (Fig. 1C), the dispersion of the valence band evolves from a single-peak to a dual-peak shape with a “dip” at the $\bar{\Gamma}$ point ($k = 0$); the $\delta = 0.0$ compound features a direct band gap in its bulk, whereas the $\delta = 1.0$ compound has a slightly indirect gap. The overall experimental evolution of the spin-orbit ground state is shown in Fig. 2A.

Now we systematically study the end product of the transition, $\text{BiTl}(\text{S}_0\text{Se}_1)_2$, and explore its spin and polarization properties far away from the Dirac node where nonlinear spin-orbit terms are also important. Such terms correspond to analogs of cubic and higher-order Dresselhaus effects arising from the symmetry of the crystal potential. Without these effects, the surface states form a cone that is isotropic in momentum space (k_x, k_y) and hence generates a circular Fermi contour (2). Spins tangentially arranged on such a circular Fermi surface lead to a Berry's phase of π , which is also a measure of the topological invariant and the axion angle ($\nu_0 = 1 = \theta/\pi$) of the system (1, 4, 6). However, the details of the symmetry and bulk crystal potential can strongly deform the surface states from that of a circle. We explored

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the Fermi contour of $\text{BiTl}(\text{S}_0\text{Se}_1)_2$ over a large energy range. This sample features an almost perfectly hexagonal Fermi surface at its native (as-grown) Fermi level (binding energy $E_B = 0.01$ eV), whereas the constant energy contour reverts to isotropic circular shapes when approaching the Dirac node ($E_B = 0.25$ eV and $E_B = 0.50$ eV). A more extreme example is n-type bulk doped Bi_2Te_3 , a topological insulator that features a highly warped concave-in snowflake-shaped Fermi surface because of its large k_F and a small band gap (25). To understand the relationship between these Dirac cone deformations and the topological invariants and axion angles (ν_0 and θ), we used spin-angle-resolved photoemission spectroscopy (spin-ARPES) (26, 27). Such spin-resolved study is also critical because Hall transport data are now possible that rely heavily on surface state topology and spin configuration data for interpretation (15).

We have carried out spin polarization texture measurements over a large range of binding

energies to capture the nonlinear regime that can be accessed by gating for transport measurements. For simplicity, we show results for the hexagonal Fermi surface above the Dirac node of $\text{BiTl}(\text{S}_0\text{Se}_1)_2$ (Fig. 2, B and E). Figure 2F shows the measured out-of-plane spin polarization (P_z) of cuts C and E (binding energy and momentum direction are defined in Fig. 2, D and E, respectively). No significant out-of-plane spin polarization component is observed within the experimental resolution for cuts such as these. The in-plane measurements, on the other hand, show large polarization amplitudes (Fig. 3A), suggesting that the spin texture is mostly 2D. On the basis of these data, we determine the direction of full 3D spin vectors following a two-step routine (25, 28). Because $P_z = 0$ (Fig. 2F), the out-of-plane polar angles are all close to 90° , so only in-plane azimuthal angles are shown in Fig. 3B. On the hexagonal Fermi contour located above the Dirac

node (Fig. 3B), the spin vectors obtained from the polarization measurements show that the ground state features a 2D in-plane left-handed chirality spin vortex (a skyrmion in momentum space; Fig. 3, B and C). The direction of the spin is roughly perpendicular to the momentum space track that connects the $\bar{\Gamma}$ point and the momentum point location of the spin on the Fermi surface, rather than being tangential to the Fermi contour, as would be expected for an ideal Dirac cone. Our data also show that the spin texture below the Dirac node is also vortex-like but features right-handed chirality. Therefore, when the system is chemically tuned through the zero carrier density (Dirac node), the chirality of the spin vortex gets inverted, as seen in the data. A systematic method of surface chemical potential tuning has been demonstrated in (6) and is also applicable here (25). However, it is not physically possible to realize this chirality inversion or chemical tuning in Bi_2Te_3 , because the node is buried under other bands.

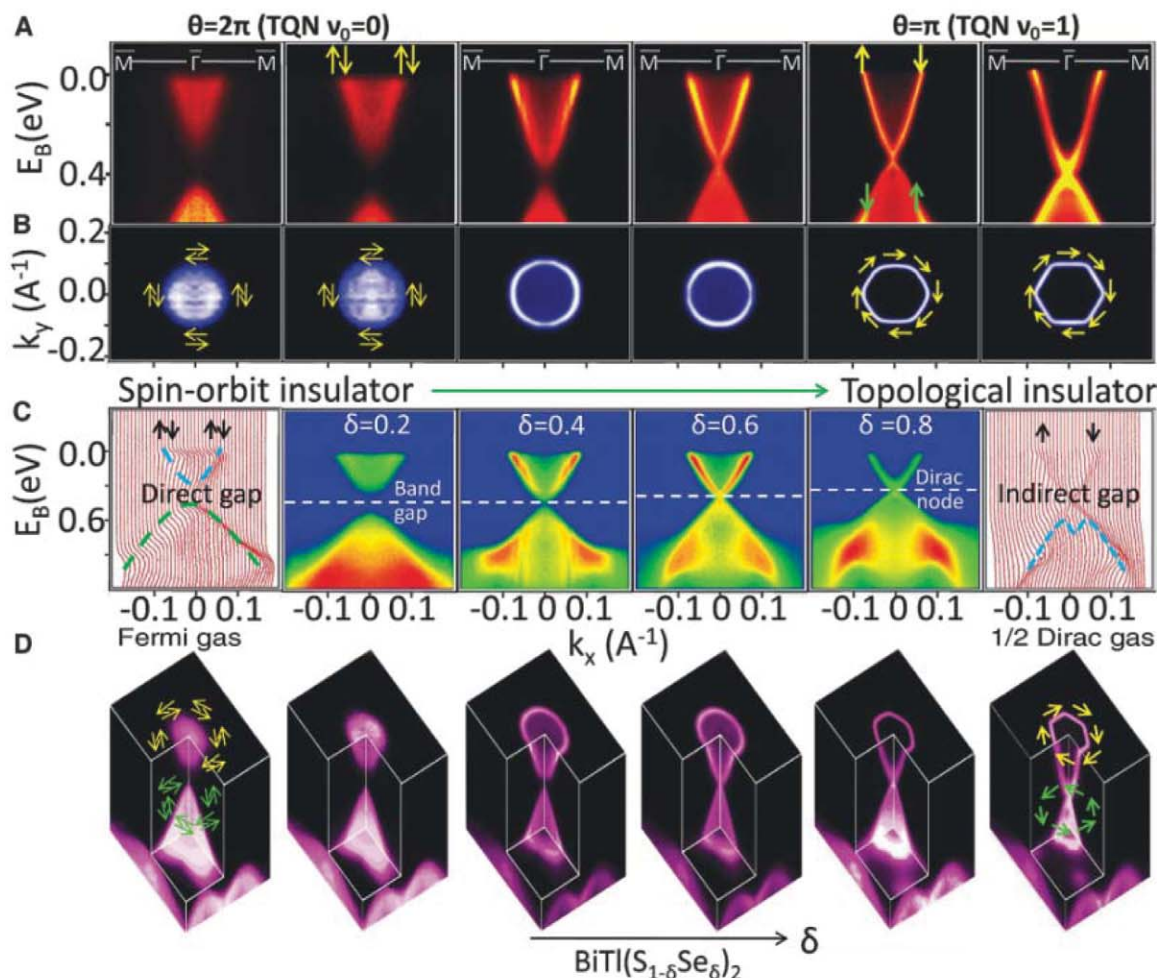


Fig. 1. Topological phase transition. (A) High-resolution ARPES dispersion maps along the $\bar{\Gamma}$ - $\bar{M}$ momentum space line, from a spin-orbit band insulator (left panel) to a topological insulator (right panel). Band insulators and topological insulators are characterized by $\theta = 2\pi$ and $\theta = \pi$, respectively. Topological quantum numbers (TQN) are denoted by topological invariant ν_0 . (B) ARPES-mapped native Fermi surfaces for different chemical compositions (from left to right, $\delta = 0$ to $\delta = 1$). (C) Left and right panels: Energy distribution curves for

stoichiometric compositions $\delta = 0$ and $\delta = 1$, respectively. Center panels: ARPES spectra indicating band gap and Dirac node for compositions $\delta = 0.2$ to $\delta = 0.8$. (D) Evolution of electronic ground state (3D band topology) imaged over a wide range of energy (vertical axis), spin (arrows), and momentum (horizontal plane). Spin textures are indicated by yellow arrows above the Dirac node and green arrows below the Dirac node. Each arrow represents the net polarization direction on a k -space point on the corresponding Fermi surface.

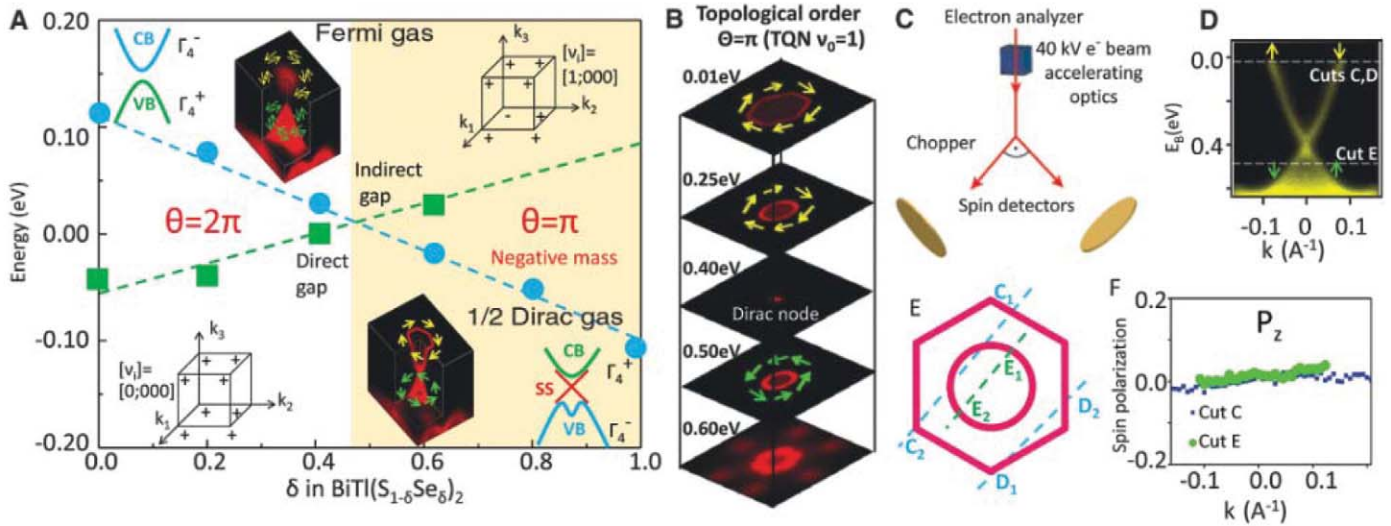


Fig. 2. Evolution of spin-orbit ground state and spin texture. **(A)** Energy levels of Γ_4^- (blue circles) and Γ_4^+ (green squares) bands, obtained from ARPES measurements as a function of composition δ . CB, conduction band; VB, valence band. Parity eigenvalues (+ or -) of Bloch states (25) are shown. The topological invariants, v_i , obtained from the parity eigenvalues are presented as $[\theta/\pi = v_0; v_1 v_2 v_3]$ where $\theta = \pi v_0$ is the axion angle and v_0 is the strong invariant (1–4). **(B)** Fermi surface topology evolution of $\text{BiTl}(\text{S}_{1-\delta}\text{Se}_\delta)_2$ across the Dirac node. The corresponding binding energies of constant-energy contours are indicated. Observed spin textures are schematically indicated by arrows.

(C) Experimental scattering geometry used to measure the spin polarization components shown in **(B)**. **(D)** ARPES-measured dispersion along the $\bar{\Gamma}-\bar{M}$ momentum space cut. The binding energies used for the cuts are as follows: E_B (cuts C and D) = 0.01 eV, E_B (cut E) = 0.50 eV. **(E)** A map of the momentum space cuts C, D, and E across the Fermi surfaces for the spin polarization measurements. The hexagonal Fermi surface is located 0.40 eV above the spin degenerate node; the circular Fermi surface is located 0.1 eV below the spin degenerate node. **(F)** Measured out-of-plane spin polarization profile of cuts C and E, showing only weak modulations.

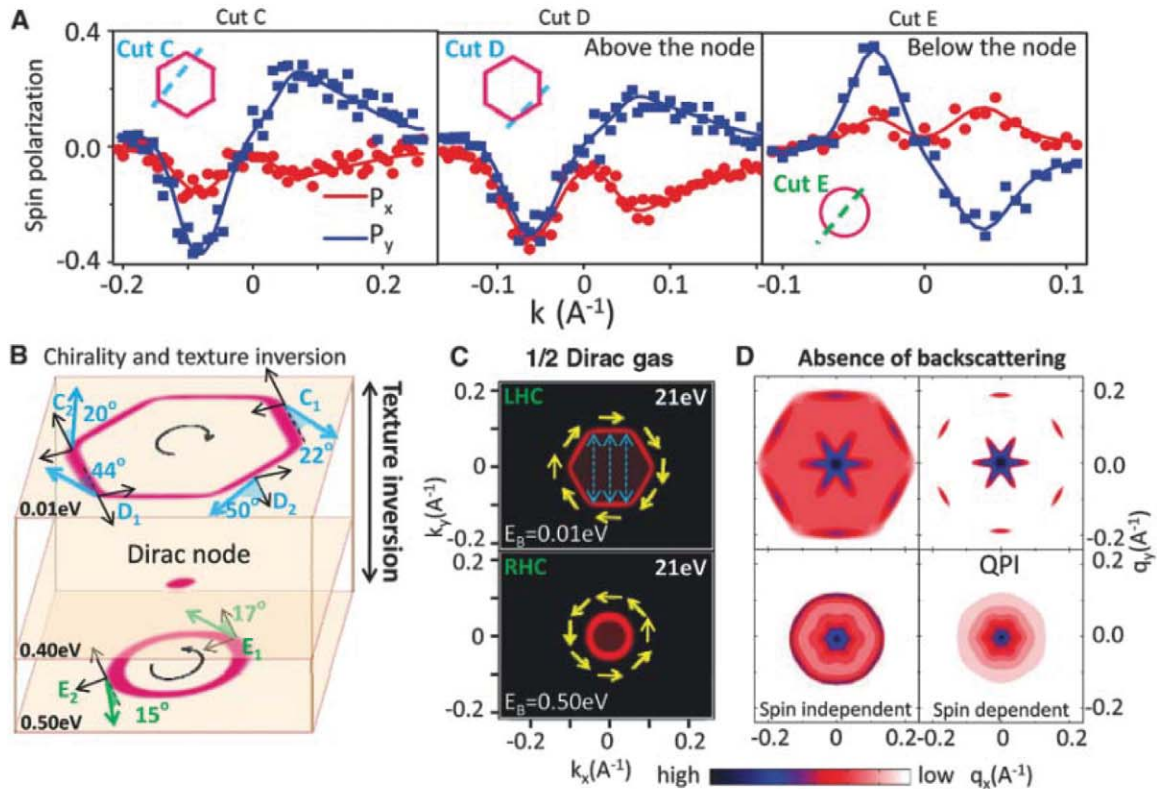


Fig. 3. Chirality inversion and spin-dependent scattering profiles for charge and spin transport. **(A)** Measured in-plane spin polarization profiles of cuts C, D, and E (Fig. 2). **(B)** Fitted in-plane azimuthal angle values of spin vectors measured along cuts C, D, and E on the ARPES-measured Fermi surfaces. Spin rotation handedness or chirality changes from left-handed (LHC) to right-handed (RHC) in passing through the Dirac node toward the higher binding energy. The binding energies are indicated at the lower left corner for the

experimental Fermi surfaces presented. **(C)** ARPES-measured Fermi surfaces are shown with spin directions based on polarization measurements. Photon energy used for spin-resolved measurements is indicated at the upper right corners. Spin texture reveals a 1/2 Dirac gas. **(D)** Left panels: Spin-independent scattering profiles on Fermi surfaces in **(C)**; right panels: Spin-dependent scattering profiles on Fermi surfaces in **(C)** relevant for surface quasi-particle transport, which is sampled by the quasi-particle interference (QPI) modes.

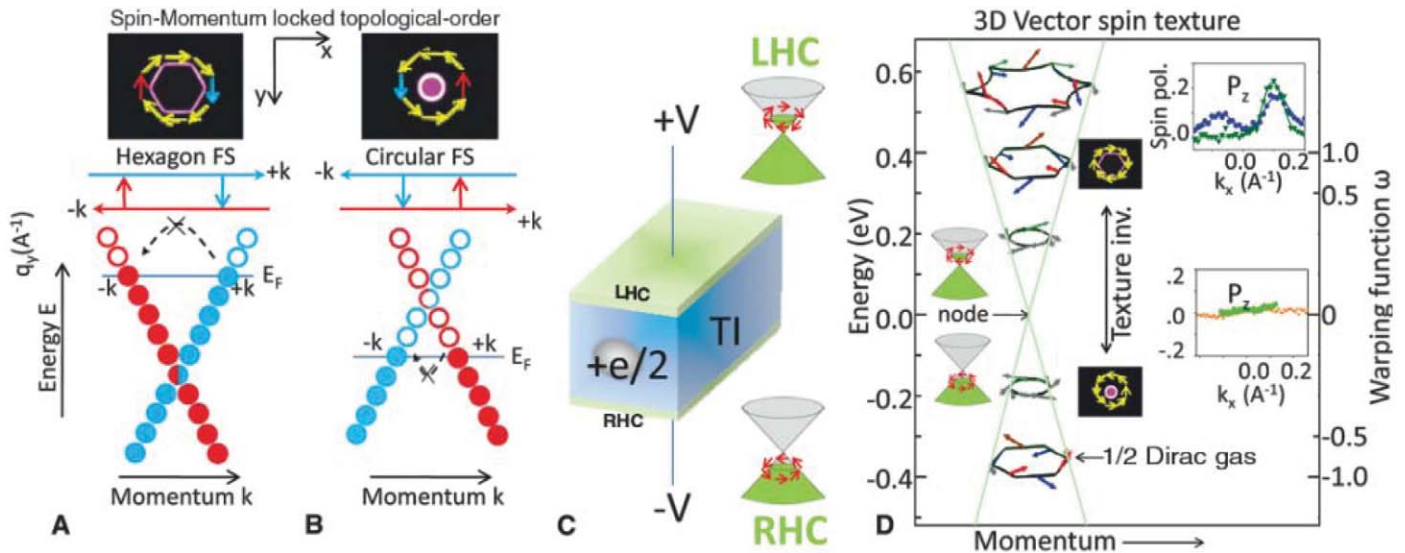


Fig. 4. Inversion of spin momentum–locking profile and spin texture phase diagram. **(A and B)** A 180° turnaround of the spin momentum–locking profile is observed when chemical potential moves across the Dirac node. In the present experiment, this is equivalent to measuring the profile above and below the Dirac node. The profiles above and below the Dirac node are shown in **(A)** and **(B)**, respectively. **(C)** A capacitor-like device geometry, using topological insulator thin films of opposite chirality (LHC and RHC) composite, is predicted to host exotic exciton properties where quasi-particle excitation carries fractional charge. **(D)** Spin texture evolution of topological surface

bands as a function of energy away from the Dirac node (left axis) and geometrical warping factor ω (right axis). The warping factor is defined as

$$\omega = \frac{k_F(\bar{r} - \bar{m}) - k_F(\bar{r} - \bar{\kappa})}{k_F(\bar{r} - \bar{m}) + k_F(\bar{r} - \bar{\kappa})} \times \frac{2 + \sqrt{3}}{2 - \sqrt{3}} \text{ where } \omega = 0, \omega = 1, \text{ and } \omega > 1 \text{ imply circular,}$$

hexagonal, and snowflake-shaped Fermi surfaces, respectively. The sign of ω indicates texture chirality for LHC (+) or RHC (-). The insets show out-of-plane 3D spin polarization measurements at corresponding Fermi surfaces.

The observed chirality inversion of the surface spin texture indicates a 180° turnaround of the spin momentum–locking profile in moving chemical potential across the Dirac node (Fig. 4, A and B). Above the Dirac node, a quasi-particle moving in the $+k$ ($+\hat{x}$) direction is locked with $+\hat{y}$ spin polarization state, whereas below the node, the $+k$ moving quasi-particle state is locked with $-\hat{y}$ spin. These spin polarization states locked to specific momentum states open up many new possibilities for electrical manipulation of spin in a topological device. Manipulation of the Fermi level of a topological insulator—for example, through electrical gating, band structure engineering, or chemical doping–induced gating (6, 14, 29)—would allow direct observation of the consequences of chirality inversion in the quasi-particle dynamics and interference (Fig. 3).

One particularly interesting proposal involves electrical or chemical gating of both top and bottom surfaces of a topological insulator thin film, resulting in a left-handed chirality (LHC) electron-like Fermi surface on the top and a right-handed chirality (RHC) hole-like Fermi surface of Dirac surface states on the bottom. The interaction between LHC and RHC surface states will lead to excitons at low temperatures with topological properties; the detection of such excitons would allow measurement of the topologically protected fractionalized charge of $\pm e/2$, which can be implemented by optical methods on a device that incorporates the texture inversion (17) possible within this materials class (Fig. 4).

To show how these spin textures are relevant in understanding and interpreting quasi-particle

transport, such as that observed in the anomalous Hall experiments (15) involving the topological insulator surfaces, we explore the key scattering processes on the hexagonal and circular Fermi surfaces observed in our data. Scattering profiles (Fig. 3D) estimated on the basis of the measured spin-ARPES Fermi surfaces and the topological spin textures of $\text{BiTl}(\text{S}_0\text{Se}_1)_2$ yield the probability of an electron being scattered in momentum transfer (scattering vector) $\mathbf{q}$ space (25, 30–32). Scattering vector $\mathbf{q}$ is defined as the momentum transfer from one point on a Fermi surface ($|\bar{k}_F\rangle$) to another ($|\bar{k}_F'\rangle$); thus, $\mathbf{q} = |\bar{k}_F'\rangle - |\bar{k}_F\rangle$. We consider the spin texture at a particular binding energy and estimate the spin-independent and spin-dependent scattering profiles (Fig. 3D). The resulting spin-dependent scattering shows a suppression of elastic back-scattering on the $\text{BiTl}(\text{S}_0\text{Se}_1)_2$ surface consistent with its topological order (25). Interestingly, for the hexagonal case, a six-fold symmetric quasi-particle scattering profile is seen, indicating the possibility for a fluctuating spin density wave-type magnetic instability that connects the parallel pieces of the observed spin-polarized Fermi surface (Fig. 3, C and D). This surface magnetic instability is expected to contribute strong fluctuations in the spin transport channel. A recent paper based on mean-field Zener theory showed that a helical magnetic order (HMO) is expected if the surface state topology is in the 3D vectorial spin texture (Fig. 4D) regime; the system can then achieve unusually high critical temperature, even up to $T_{\text{HMO}} \approx 100$ K (30). Topological quantization in such a system might then survive at a temperature

several orders of magnitude higher than in conventional quantum Hall systems.

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Orbital-Independent Superconducting Gaps in Iron Pnictides

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The origin of superconductivity in the iron pnictides has been attributed to antiferromagnetic spin ordering that occurs in close combination with a structural transition, but there are also proposals that link superconductivity to orbital ordering. We used bulk-sensitive laser angle-resolved photoemission spectroscopy on $\text{BaFe}_2(\text{As}_{0.65}\text{P}_{0.35})_2$ and $\text{Ba}_{0.6}\text{K}_{0.4}\text{Fe}_2\text{As}_2$ to elucidate the role of orbital degrees of freedom on the electron-pairing mechanism. In strong contrast to previous studies, an orbital-independent superconducting gap magnitude was found for the hole Fermi surfaces. Our result is not expected from the superconductivity associated with spin fluctuations and nesting, but it could be better explained invoking magnetism-induced interorbital pairing, orbital fluctuations, or a combination of orbital and spin fluctuations. Regardless of the interpretation, our results impose severe constraints on theories of iron pnictides.

The high-transition-temperature (T_c) superconducting iron pnictides (*I*) of the BaFe_2As_2 ($\text{Ba}122$) family exhibit a typical phase diagram (Fig. 1A) in which the parent compound remains metallic through an antiferromagnetic (AF) spin-ordering transition at T_N and a tetragonal-to-orthorhombic structural transition at T_S (2, 3). Both T_N and T_S decrease in a similar fashion upon ion substitution and lead to an emergent dome-shaped superconducting (SC) phase with a high T_c of up to 55 K (4). Such high T_c s are not expected from a conventional electron pairing through lattice vibrations (5). Focusing on the nesting tendency connecting quasi-cylindrical Fermi surfaces (FSs), electron pairing mediated by the AF spin fluctuations has been argued (6, 7).

On the other hand, there is a proposal that orbital ordering (8–10), which causes inequivalency of the Fe *3d* partial density of states, occurs at T_S . This phenomenon could give rise to C2 symmetric physical properties that cannot be expected from only 1% lattice distortion at T_S . In particular, inequivalent occupation of the *xz* and *yz* orbitals (*x*, *y*, and *z* represent orthorhombic axes) near the Fermi level (E_F), as observed by angle-resolved photoemission spectroscopy (ARPES), indicates the potential role of orbital degrees of freedom (11). Based on the multiorbital nature of iron pnictides, SC pairing through orbital fluctuations has been proposed (12–15).

Experimental evidence has favored different SC pairing symmetries. Sign-reversal superconductivity suggested by scanning tunneling microscopy on $\text{Fe}(\text{Te},\text{Se})$ (16) and inelastic neutron spectroscopy on $(\text{Ba},\text{K})\text{Fe}_2\text{As}_2$ (17) is consistent with the $s\pm$ wave symmetry expected from spin fluctuations. The robustness of T_c against the impurities in $\text{LaFeAs}(\text{O},\text{F})$ (18) suggests the $s++$ wave superconductivity, which may be caused by orbital fluctuations. Such material dependence in pairing symmetry can also arise from the balance of these two pairing mechanisms because a crossover from $s\pm$ to $s++$ symmetry may occur, depending on the strengths of microscopic electronic parameters (12–15). However, to date there has been no experimental evidence for the role of orbital fluctuations on the superconductivity in the pnictides.

The pairing mechanism will be reflected in the momentum dependence of the SC gap properties. Spin fluctuation mechanism predicts strongly orbital-dependent SC gap magnitude (7, 19), but orbital fluctuations should eliminate such orbital dependence (13–15). Because each FS sheet in iron pnictides has a distinct d-orbital character, probing the FS dependence of the SC gap magnitude will be a crucial test for identifying the contribution of the orbital fluctuations in the electron pairing. Here, we report the FS dependence of the SC gap magnitude of representative iron-pnictide superconductors $\text{BaFe}_2(\text{As}_{0.65}\text{P}_{0.35})_2$ (AsP122) and $\text{Ba}_{0.6}\text{K}_{0.4}\text{Fe}_2\text{As}_2$ (BaK122) by using bulk-sensitive laser-ARPES (20) [see section 1 of the supporting online material (SOM)].

ARPES, using a fixed photon energy ($h\nu$), probes a specific profile of the in-plane FSs perpendicular to the momentum axis along the *z* direction k_z . Especially for AsP122 with strong FS warping, the orbital character depends on the k_z value (21). Estimation of k_z value corresponding to 7 eV photons of laser-ARPES is thus necessary for investigating the orbital dependence of the SC gap magnitude. Figure 1B is a schematic of the experimental FS profile along the k_z axis of AsP122 taken from (22). Whereas the inner hole FS is almost cylindrical, the outer one gets enlarged toward $k_z = \pi$, consistent with the band calculations (inset of Fig. 1A) (21). We used a synchrotron radiation photon source of 10 eV and 7 eV to find this characteristic warping in the inner (k_{F1}) and outer (k_{F2}) Fermi momenta. As shown in Fig. 1, C and D, separation between k_{F1} and k_{F2} in C was three-fourths of that in D, which indicates that 7 eV photons probe the FS profile at $k_z \sim \pi$ to 1.2π . This estimate of k_z for $h\nu = 7$ eV was also quantitatively confirmed by the comparable FS size obtained by the higher photon energy of 63 eV probing $k_z \sim \pi$ (22) (Fig. 1E).

High-resolution laser-ARPES *E-k* images for AsP122 (Fig. 2, B and C) are along cut 1 and 2 indicated in Fig. 2A, respectively. Both the inner and middle hole bands were resolved as the two-peak feature in the momentum distribution curve (MDC) near E_F (Fig. 2B), although they are nearly degenerate in Fig. 1, C and D. We could separate three hole bands along cut 1 and 2. The temperature (*T*) dependence of the energy distribution curves (EDCs) at k_F in cut 2 (outer FS) shows the SC gap opening below bulk T_c (Fig. 2D), and the SC gap magnitude Δ was directly extracted from the fitting procedure using a Bardeen-Cooper-Schrieffer (BCS) spectral function (SOM, section 2). This Δ value is in excel-

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lent agreement with the BCS-like T dependence (inset of Fig. 2D). Figure 2, E to G, shows EDCs in the SC state and fitting results for each hole FS. A shift in the leading edge relative to E_F indicates an SC gap opening. As shown in Fig. 2H, the FS angle dependence of Δ indicates the fully gapped nature of the three hole FSs in AsP122. Considering the nodal SC gap symmetry proposed by the magnetic penetration depth, thermal conductivity (23) and nuclear magnetic resonance measure-

ments (24), our results rule out d-wave symmetry and imply the possible presence of line nodes in electron FSs around zone corners. The Δ of each hole FS is comparable to the common reduced gap value of $2\Delta/k_B T_c \sim 3.0$.

The laser-ARPES results on BaK122 (Fig. 3A) showed three hole FSs around the zone center, which is consistent with the first-principles band calculations with experimental lattice constants. We separately observed the three hole bands by

varying the laser polarizations. Figure 3B shows the three hole bands measured by circular polarization along the momentum cut represented by the white arrow in Fig. 3A. When we chose p-polarization, the band A became dominant (Fig. 3C), in contrast to the band B and C being active in s-polarization (Fig. 3D). Such strong polarization dependence of the photoelectron intensity implies a different d-orbital character in these hole bands (17). We make use of the

Fig. 1. (A) Schematic phase diagram of $\text{BaFe}_2(\text{As,P})_2$ and $(\text{Ba,K})\text{Fe}_2\text{As}_2$. T_N represents the AF transition temperature and T_S a tetragonal to orthorhombic structural transition temperature. Red arrows indicate stripe-type AF spin ordering on the orthorhombic lattice. Inset shows calculated FSs of optimally doped $\text{BaFe}_2(\text{As,P})_2$. (B) Momentum cut along k_z axis corresponding to the gray rectangle in the inset of (A). FS profile of $\text{BaFe}_2(\text{As,P})_2$ reported in (22) is indicated by the gray curve. (C and D) Hole band dispersions around the zone center measured by synchrotron radiation photon sources of $h\nu = 10$ eV and 7 eV, respectively. Inner (k_{F1}) and outer (k_{F2}) Fermi momenta are estimated by plotting the peak positions of MDCs below E_F . Comparing the k_F dispersions along the k_z axis (22), momentum cuts measured by $h\nu = 7$ eV and 10 eV are superimposed as black curves at the bottom of (B). (E) Quantitative comparison of the k_F measured by $h\nu = 7$ eV and 63 eV, indicating reliable determination of $k_z = \pi$. Black circles represent k_{F1} (nearly degenerate inner and middle hole bands in Fig. 2B) and k_{F2} (outer hole band in Fig. 2C) obtained by synchrotron radiation photon source of $h\nu = 7$ eV. The gray curve represents FSs obtained by $h\nu = 63$ eV (22).

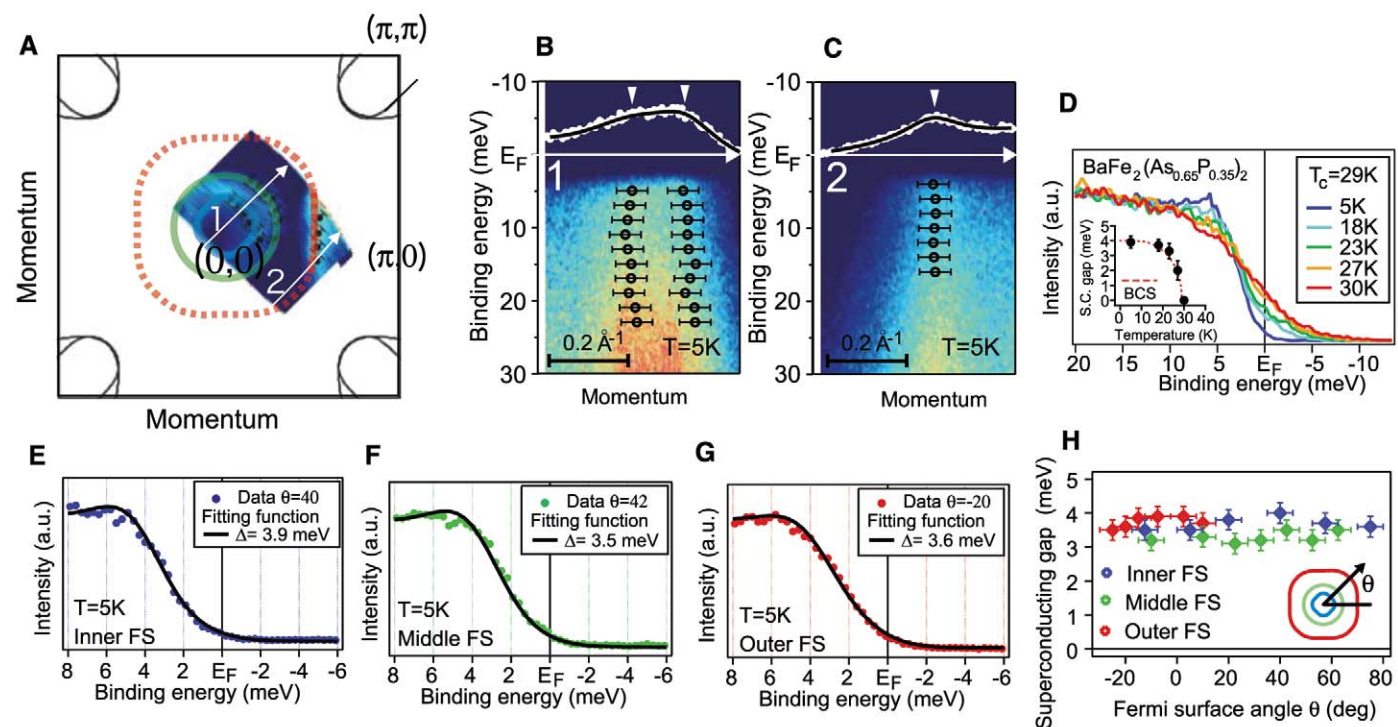
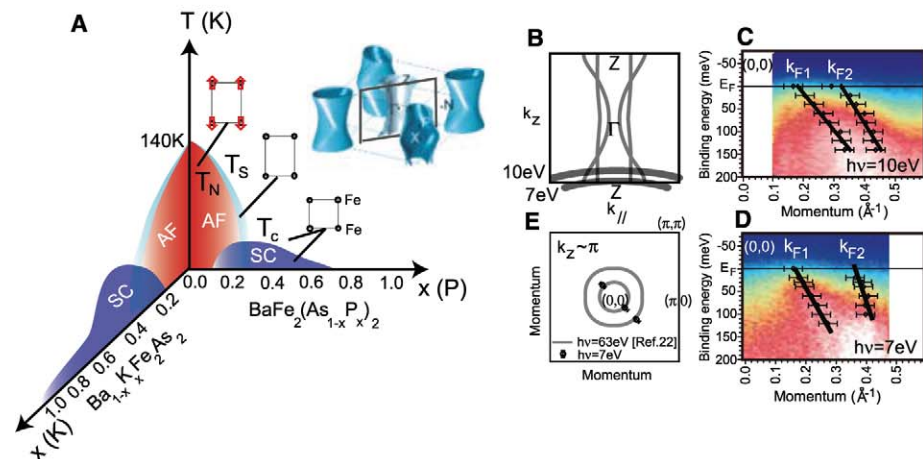


Fig. 2. Laser-ARPES on $\text{BaFe}_2(\text{As}_{0.65}\text{P}_{0.35})_2$. (A) FSs around the (0,0) point of $\text{BaFe}_2(\text{As}_{0.65}\text{P}_{0.35})_2$, measured by circularly polarized laser. (B and C) Band dispersions measured by circular polarization along cut 1 and cut 2 in (A), respectively. (D) T dependence of EDC at k_F in cut 2. Inset shows T dependence of the SC gap magnitude determined by the fitting procedure,

using the BCS spectral function. The red broken curve represents BCS-like T dependence. (E to G) Fitting results on the EDCs in the SC state measured at k_F of inner, middle, and outer FS, respectively. (H) FS angle dependence of the SC gap magnitude in each hole FS. Inset shows the definition of the FS angle θ .

polarization dependence for separating band A from others to discuss its T dependence across T_c .

In Fig. 3E, we show the T dependence of the EDC at k_F of band A measured with a p-polarized laser. A distinct two-peak structure (peak 1 and peak 2) was observed near E_F with decreasing T . To emphasize the peak 1' in the unoccupied state, we divided EDCs using a Fermi Dirac (FD) function convoluted by a Gaussian corresponding to the experimental energy resolution. Divided spectra were further normalized by that measured at 60 K (Fig. 3F). Both peaks 1 and 1' appeared just below bulk T_c , accompanying a gap structure centered at E_F consistent with the Bogoliubov quasiparticle picture. This observation strongly suggests that peaks 1 and 1' arise from superconductivity. However, peak 2 is found even in the normal state, which suggests that it is a non-SC peak (for its detailed analysis and possible origin, see SOM section 3).

We can quantitatively estimate Δ for each hole FS sheet focusing on the peak 1 and gap structure around E_F in a similar manner to that used for AsP122. By extracting the SC gap magnitude from a series of T -dependent EDCs in Fig. 3E, an excellent agreement with BCS-like T dependence is obtained as shown in Fig. 3G. Figure 3, H to J, shows the EDCs in the SC state and the fitting results for each hole FS in BaK122. As indicated by the leading-edge shift in EDCs at FS angles $\theta = 8$ and $\theta = 45$ degrees (Fig. 3H), we observed slight anisotropy in Δ . The definition of the FS angle θ and the gap anisotropy in each hole FS is summarized in Fig. 3K. In addition to the fully gapped nature, we found comparable Δ values for the three hole FSs with a small reduced gap value of $2\Delta/k_B T_c \sim 1.7$ (SOM, section 4). This result is in a strong contrast to the “two-gap” picture previously observed at around zone center (25, 26) (SOM, section 5).

The FS-sheet dependences of SC gap magnitude in AsP122 and BaK122 obtained by laser-ARPES are summarized in Fig. 4. The most remarkable feature commonly observed in AsP122 and BaK122 is the coincidence of Δ in the three hole FSs. At least for AsP122, the size and this characteristic behavior in SC gaps are observed independently of the k_z values (SOM, section 6). First-principles band calculations indicate that each hole FS is characterized by a different orbital character. This property was also experimentally confirmed by the strong polarization dependence in hole bands of BaK122 (Fig. 3, B to D). The comparable Δ for all hole FSs thus indicate that they are almost orbital-independent.

Of course, conventional isotropic electron-phonon interaction can give a homogeneous Δ for all FSs, but this mechanism is not likely in iron pnictides for three reasons. First, high T_c up to 55 K (4) is not expected from the conventional electron-phonon interactions (5). Second, a nodal SC gap is experimentally suggested in several iron pnictides (23, 24, 27, 28). Third, material dependence of the SC gap magnitude implies electron-pair scattering between hole and elec-

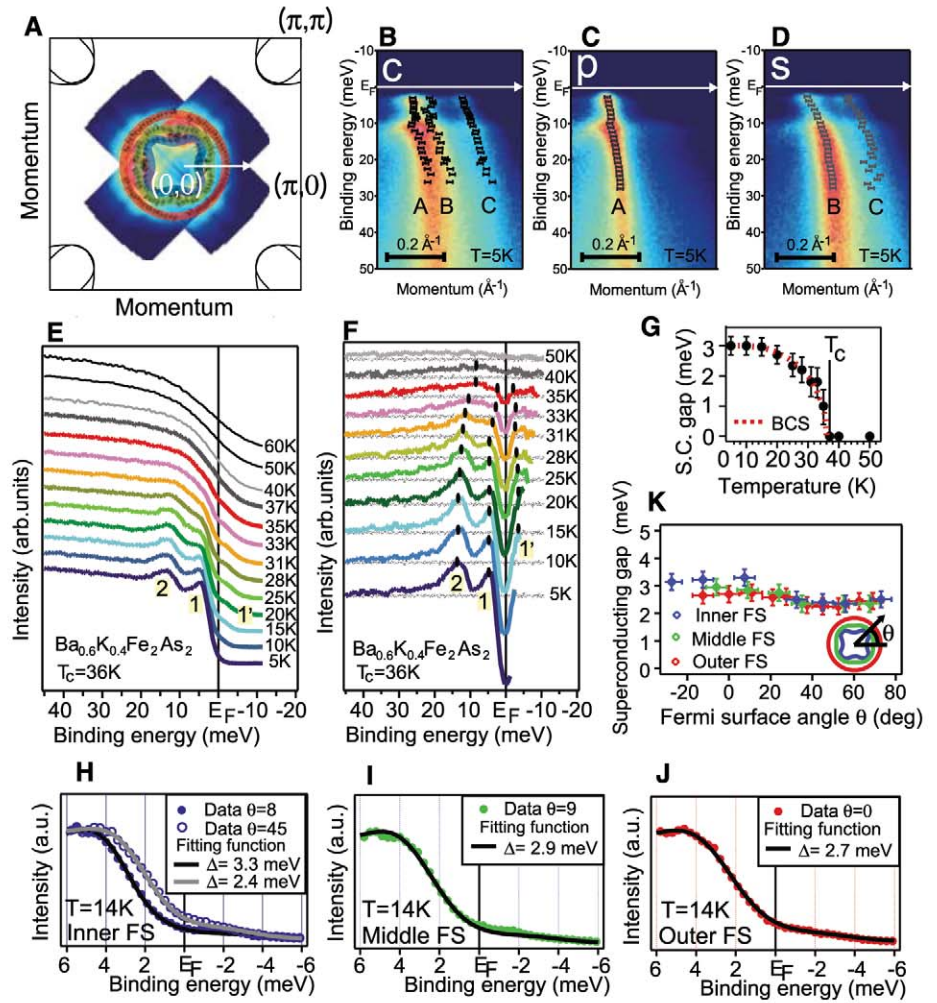


Fig. 3. Laser-ARPES on $\text{Ba}_{0.6}\text{K}_{0.4}\text{Fe}_2\text{As}_2$. (A) FSs around the (0,0) point of $\text{Ba}_{0.6}\text{K}_{0.4}\text{Fe}_2\text{As}_2$, measured by circularly polarized laser. (B to D) Band dispersions measured by circular, p-, and s- polarized laser, respectively. The corresponding momentum cut is shown by the white arrow in (A). (E) T dependence of EDC at k_F of band A, measured by p-polarization. The contribution from other hole bands is already removed by choosing polarization, and the spectral weight at E_F is very low, indicative of the clear SC gap opening. (F) EDCs divided by FD functions. They are further normalized by the divided EDC at 60 K to extract the T -dependent component. Black dots represent the peak position of peak 1, peak 1' and peak 2. (G) T dependence of the SC gap magnitude estimated by fitting analysis using the BCS spectral function. The red broken curve represents BCS-like T dependence. (H to J) Fitting results on the EDCs in the SC state, measured in inner, middle, and outer FS, respectively. (K) FS angle dependence of the SC gap magnitude in each hole FS. Inset shows the definition of the FS angle θ .

tron FSs, originating in the FS nesting (SOM, section 7). We expect that the pairing mechanism is not purely of phonon mediation but is closely related to the spin fluctuations and/or orbital fluctuations. For a spin-fluctuations pairing mechanism (6, 7, 19), the SC gap is most closely related to the spin susceptibility. The calculated spin susceptibility suggests dominant electron-pair scattering between disconnected FSs composed of the same orbital character (7, 19) (intraorbital pairing), as illustrated in Fig. 4B. Then, Δ is expected to be highly sensitive to the orbital character, depending on the overlap of the FS shape it composes. This property, nevertheless, contradicts the orbital-insensitive SC gaps we observed in the laser-ARPES experiments.

In particular, we focus on the $3z^2-R^2$ (X , Y , and Z represent tetragonal axes) orbital because it does not contribute to the electron FSs around zone corners in 122 systems (Fig. 4, C and D) (21). In this case, intraorbital electron pairing between the disconnected FSs is suppressed, and then the Δ of the $3z^2-R^2$ orbital electrons should be quite small. According to the band calculations in (21), the $3z^2-R^2$ orbital is dominant in the outer hole FS of AsP122 around $k_z \sim \pi$. This is supported by our polarization-dependent laser-ARPES, which shows a dominantly $3z^2-R^2$ orbital character in the outer hole FS of AsP122 (SOM, section 8). Taking this into account, the comparable size of SC gap magnitude for various orbital characters, including the $3z^2-R^2$ orbital,

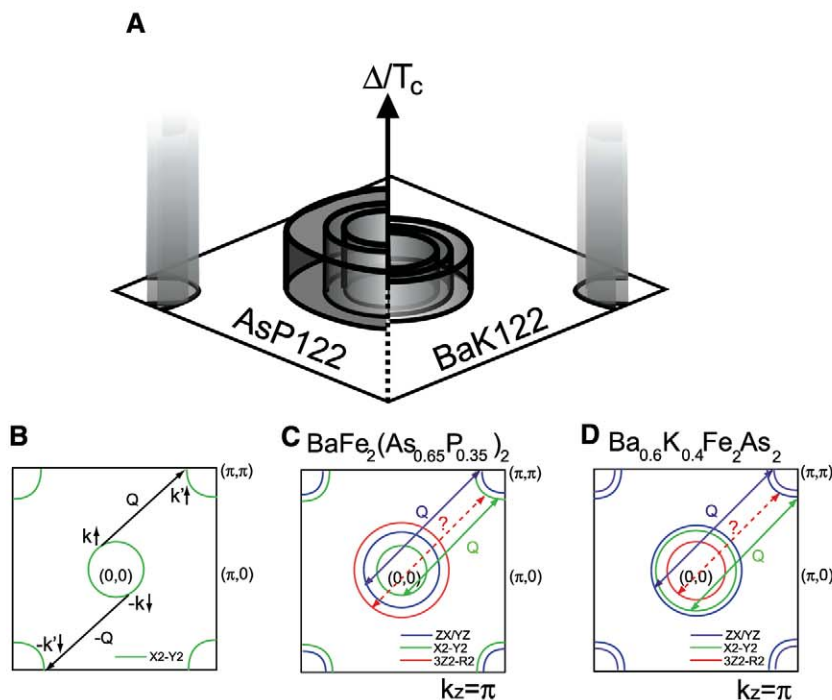


Fig. 4. (A) Schematic momentum dependence of the superconducting gap magnitude around $k_z = \pi$ in $\text{BaFe}_2(\text{As}_{0.65}\text{P}_{0.35})_2$ and $\text{Ba}_{0.6}\text{K}_{0.4}\text{Fe}_2\text{As}_2$, determined by laser-ARPES. Those in electron FSs around zone corners (light gray) are speculations. (B) Illustration of the electron pair scattering from $(k \uparrow, -k \downarrow)$ to $(k' \uparrow, -k' \downarrow)$ between disconnected FSs of the same orbital character (intraorbital pairing). Electrons are scattered by the AF spin fluctuations with the wave vector Q derived from the FS nesting. (C and D) Schematic FS profiles at $k_z = \pi$ for AsP122 and BaK122, respectively. The number of FS sheets and their orbital characters are based on the first-principles band calculations using experimental atomic positions of $x = 0.4$ ($x = 0.33$) for BaK122 (AsP122) with BaFe_2As_2 structure. FS sheets composed of ZX/YZ, X²-Y², and 3Z²-R² orbitals (X, Y, and Z represent tetragonal axes) are colored in blue, green, and red, respectively. Arrows with wave vector Q represent the nesting tendency between FS sheets of the same orbital character. Because 3Z²-R² orbital electrons do not form the FSs around zone corners, they cannot participate in electron pairing through the spin fluctuations of wave vector $\sim Q$ (15, 30).

can hardly be explained by the spin-fluctuation mechanism alone.

However, the importance of interorbital coupling (29, 30) and the role of the orbital fluctuations are being theoretically raised (12–15), which has been neglected in the genuine spin-fluctuations pairing mechanism. Such interorbital electron pairing is expected to dissolve the orbital dependence of Δ and can explain the comparable Δ around the zone center observed by laser-ARPES.

A multiorbital band structure near E_F in iron pnictides has the potential to enhance the orbital fluctuations. In addition, orbital fluctuations are expected to evolve in the vicinity of the orbital ordered state. In the parent material of iron pnictides, antiferromagnetic ordering generally occurs accompanying the structural transition from tetragonal to orthorhombic crystal symmetry (2, 3) (Fig. 1). Recently, orbital ordering at the structural transition was theoretically proposed (8–10). Previous laser-ARPES on Ba122 reported unequal occupation in the electronic structure of zx and yz orbitals below the magneto-structural transition supporting the possible orbital ordering scenario (11). In addition,

the spin and orbital degrees of freedom might be strongly coupled for the parent and superconducting compounds (31–35). Theoretical studies have shown that superconductivity in the iron pnictides could be derived from orbital fluctuations mixed with spin fluctuations (31, 35). Considering this background, the present result is reasonably explained as an indication of the importance of orbital fluctuations for the superconductivity of the iron pnictides; this should be taken into account at least on the same footing as spin fluctuations. Thus, while a dominantly orbital-fluctuation-derived superconductivity can be somewhat speculative and needs to be confirmed by further work, our results clearly provide severe constraints for a complete theory of superconductivity in the iron pnictides.

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Low-Power Switching of Phase-Change Materials with Carbon Nanotube Electrodes

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Phase-change materials (PCMs) are promising candidates for nonvolatile data storage and reconfigurable electronics, but high programming currents have presented a challenge to realize low-power operation. We controlled PCM bits with single-wall and small-diameter multi-wall carbon nanotubes. This configuration achieves programming currents of 0.5 microampere (set) and 5 microamperes (reset), two orders of magnitude lower than present state-of-the-art devices. Pulsed measurements enable memory switching with very low energy consumption. Analysis of over 100 devices finds that the programming voltage and energy are highly scalable and could be below 1 volt and single femtojoules per bit, respectively.

Phase-change materials (PCMs) such as chalcogenides like $\text{Ge}_2\text{Sb}_2\text{Te}_5$ (GST), have amorphous (a) and crystalline (c) phases with contrasting electrical and optical properties. PCMs are the active material in rewritable digital video discs (DVDs), where phase transformations are induced and read by a pulsed laser (1, 2). The data in electrically programmable PCMs are stored as changes in bit resistivity (3–6), which can be reversibly switched with short voltage pulses and localized Joule heating. In this sense, PCMs are appealing compared to other semiconductor memories where data are stored as charge and are susceptible to leakage and volatile behavior. Electrically programmable PCMs have captured wide interest for applications in nonvolatile memory (7, 8) and reprogrammable circuits (5, 6) with low voltage operation, fast access times, and high endurance (3, 4). These attributes make them contenders for a “universal” nonvolatile memory, which could replace all data storage from random-access memory to hard disks. However, a drawback of PCMs is their high programming current (>0.1 mA), because Joule heat must be coupled to a finite bit volume, previously achieved with 30- to 100-nm-diameter nanowires (9–11) or metal interconnects (12–14).

We used carbon nanotubes (CNTs) with diameters of ~ 1 to 6 nm as electrodes (15, 16) to reversibly induce phase change in nanoscale GST bits. Our findings address the potential size and power reduction that are possible for programmable bits of PCM. We demonstrate reversible switching with programming currents from 0.5 to 8 μA , two orders of magnitude lower than state-of-the-art PCM devices. We also present a device-scaling study that suggests memory switching is possible with voltages below 1 V and energy less than femtojoules per bit.

The CNTs used in this work were grown by chemical vapor deposition (CVD) with Fe catalyst particles on SiO_2/Si substrates (17, 18) [also see (19)]. We obtained single-wall and small diameter multi-wall CNTs, and we found that both can be used to switch GST bits. The as-grown CNTs span Ti/Pd (0.5/40 nm) metal contacts with 1 to 5 μm of separation (fig. S1). We then created nanoscale gaps in the CNTs through electrical breakdown (20) in air or under Ar flow, as illustrated in Figs. 1 and 2A, left inset. This simple approach yielded a wide range of nanogaps (from ~ 20 to 300 nm) in more than 100 devices, which was essential for our subsequent scaling study. The nanogap is typically near the middle of the CNT, consistent with the electrical breakdown location and with negligible Pd contact resistance (20, 21). Then, a ~ 10 -nm GST film was sputtered over the device surface (fig. S2), with settings previously found to preserve the good electrical characteristics of CNTs (18, 19). This

deposition fills the CNT nanogaps, creating self-aligned lateral PCM bits (Fig. 1). Such devices can be readily switched and examined by atomic force microscopy (AFM) (Fig. 2); however, a ~ 5 -nm SiO_2 capping layer deposited after the GST without breaking vacuum (12) is used to prolong the switching lifetimes.

Devices are initially in the off state (Fig. 1C) because the as-deposited GST films are amorphous (a-GST) and highly resistive, with resistance $R_{\text{OFF}} \sim 50$ Mohm (22). A voltage applied at the CNT contacts creates a sizable electric field (E -field) across the nanogap and switches the GST bit to the crystalline phase (c-GST), which lowers the device resistance by about two orders of magnitude to $R_{\text{ON}} \sim 0.5$ Mohm. Although a-GST covers the entire device, the switching occurs only in the nanogap, which is the location of highest E -field and Joule heating.

To test initial memory switching, we sourced current and measured voltage across the devices (Fig. 2). The amorphous bits displayed switching at a threshold voltage, V_T , as is typical with GST (7, 8), and a sharp transformation to a conductive phase under high E -field. Importantly, we note that little voltage is dropped across the CNT electrodes, which are always more conductive than the GST bit, as confirmed with finite-element (FE) simulations (19). Transport in the a-GST material is temperature-activated (23) even in the ~ 10 -nm-thin films, as shown in Fig. 2A, right inset, and discussed in (19). Once threshold switching occurs, the bit crystallizes from Joule heating, and this marks the set transition. The set current was of the order ~ 1 μA in more than 100 devices tested (19), two orders of magnitude lower than set currents in conventional PCM devices. However, the V_T scaled linearly with the nanogap size (see below). This linear relationship provides strong

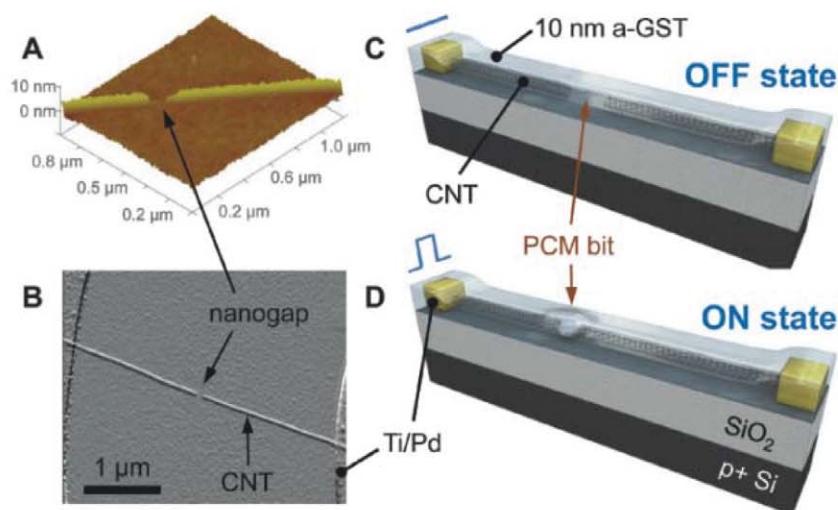


Fig. 1. Schematics of CNT-PCM device. (A) AFM imaging of nanogap created after CNT breakdown under electrical stress (20). (B) AFM image of an as-fabricated device. (C and D) Schematic of device obtained after deposition of GST thin film. The device is in its off state immediately after fabrication, with highly resistive a-GST in the nanogap. (D) The device is switched to its on state after an electric field in the nanogap transforms the bit to its conductive c-GST phase.

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evidence that threshold switching in a-GST is driven by E -field (24, 25) even at the minimal bit sizes explored here.

We examined reversible switching of our devices through pulsed measurements. In Fig. 3A, we plotted the resistance after a series of pulses

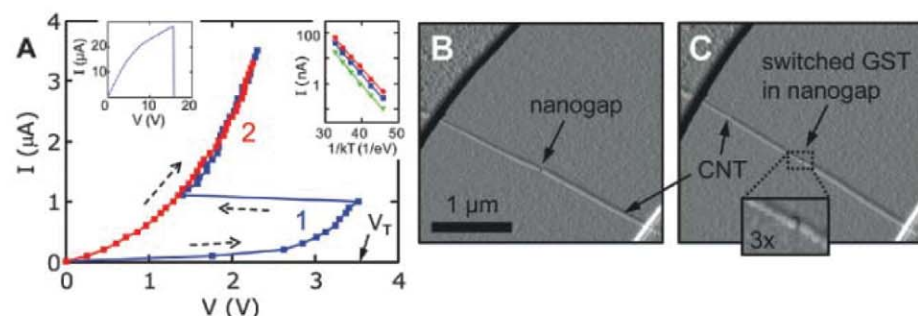


Fig. 2. Initial antifuse-like switching. **(A)** Current-voltage of a device with CNT diameter ~ 3 nm, nanogap ~ 35 nm, and GST film thickness ~ 10 nm. The initial sweep (no. 1) turns the bit on (a $\rightarrow$ c) at ~ 1 μ A and $V_T = 3.5$ V. The c-GST bit phase is subsequently preserved (no. 2). The left inset shows the current-voltage of the CNT as used to create the nanogap before GST deposition (20). The right inset shows temperature-activated transport in the subthreshold regime after a-GST deposition [also see fig. S9 (19)]. The activation energy ~ 0.38 eV decreases slightly with voltage (19), consistent with trap-assisted transport in disordered a-GST (23). **(B and C)** AFM images of the same device before and after switching. Small changes of GST volume in the gap can be seen after switching here without a capping layer (18). Also see fig. S6 in (19).

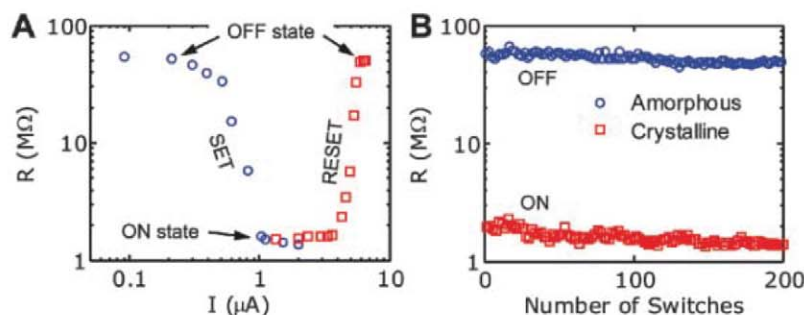


Fig. 3. Reversible memory operation using pulsed measurements. **(A)** Device resistance versus current pulse magnitude. The width of the set and reset pulses are 150 ns (20-ns falling edge) and 50 ns (8-ns falling edge), respectively, as limited by our experimental setup. Sharp transitions are seen at 1 μ A (set) and 5 μ A (reset) current, two orders of magnitude lower than the present state of the art (9–14). **(B)** Memory endurance test showing excellent separation between on and off states, with no degradation after hundreds of cycles (set pulse, 1.5 μ A and 150 ns; reset pulse, 6.0 μ A and 50 ns). The device shown here is covered by the ~ 5 -nm SiO_2 capping layer.

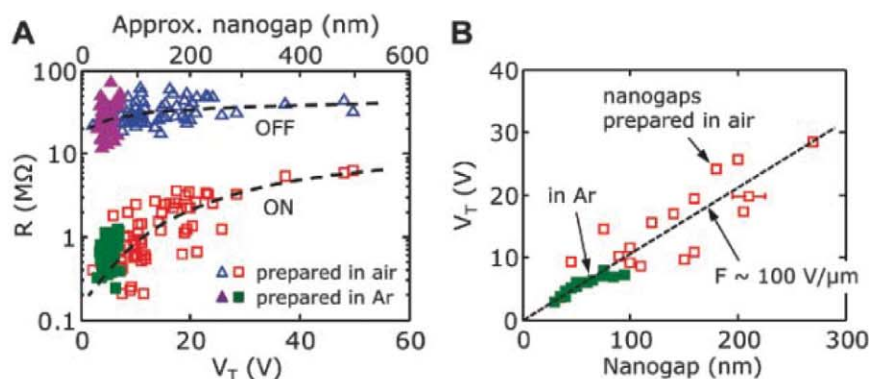


Fig. 4. Scaling trends of memory devices. **(A)** On- and off-state resistance for 105 devices shown versus V_T . As marked, 61 nanogaps were created in air ambient (open symbols); the other 44 devices were formed under Ar flow (solid symbols). Ar-formed nanogaps are consistently smaller (<100 nm) and yield lower-power devices. Dashed lines are trends to guide the eye. **(B)** Threshold voltages scale proportionally to size of nanogap, at an average field of ~ 100 V/ μ m. The dashed line is a linear fit, indicating excellent device scalability. Lateral error bar is estimated uncertainty from nanogap measurement under AFM.

with the same duration (150 ns) and increasing amplitude, starting from the resistive off state. The resistance decreases abruptly when the current exceeds ~ 1 μ A, marking the set transition. As in Fig. 2, this signals the transformation of GST in the nanogap to the c-phase, thus “reconnecting” the two CNT electrodes. The resistance increases again when the current exceeds ~ 5 μ A, which is the reset transition. This behavior is consistent with fast melting and quenching of the bit (7), returning the material to the a-GST phase. Repeated cell switching (Fig. 3B) exhibited good stability after several hundred cycles in devices capped with SiO_2 , as described above.

The dimensions of the bits examined here are in general defined by the small nanogaps (down to ~ 20 nm), the thin (~ 10 nm) GST film, and the CNT electrode diameters (~ 1 to 6 nm). The low thermal conductivity of GST (19) appears to play a role in laterally confining the bit to a scale not much greater than the CNT diameter. The small lateral extent of the bits can be seen in Fig. 2C and fig. S6 and also confirmed with simulations (19, 26). We estimate the effective bit volumes addressed here are as small as a few hundred cubic nanometers.

We present a statistical study of more than 100 devices in Fig. 4. First, we plot R_{ON} and R_{OFF} versus their respective threshold voltage V_T in Fig. 4A, showing two distinct memory states for every device studied. During fabrication, 61 of the CNT nanogaps were created in air and 44 were created under Ar flow, the latter producing smaller gaps because of reduced oxygen (15, 19). We note R_{OFF} values are fairly constant (22). However, R_{ON} scales proportionally with V_T , as seen in Fig. 4A, because both R_{ON} and V_T are related to the nanogap size. R_{ON} is dominated by the resistance of the c-GST and proportional to the nanogap size, because the CNT electrodes are much more conductive. The nanogap size also determines V_T , because threshold switching in a-GST is driven by the E -field in the nanogap. The linear scaling trend between V_T and nanogap size in Fig. 4B supports this observation, with an average threshold field of ~ 100 V/ μ m. This value is comparable to ~ 56 -V/ μ m threshold field measured in 30-nm GST films (27) and an order of magnitude lower than the breakdown field of SiO_2 (28), indicating the switching indeed occurs in the GST bit. The mean set currents across all nanogaps fabricated in air and Ar were nearly identical at ~ 2 μ A, with a range of 0.5 to 4 μ A (fig. S7C). Reset currents were typically four times higher, ranging from 5 to 8.5 μ A as shown in Fig. 3 and fig. S7D.

We comment on the ultimate scaling limits of such materials and technology. For our “best” results, switching occurred at <1 μ A (set), ~ 5 μ A (reset), and ~ 3 V across 20- to 30-nm nanogaps, with only a few microwatts of programming power. The programming current and power are two orders of magnitude lower than present state of the art (12–14), enabled by the very small volume of PCM addressed with a single CNT.

The minimum energy per bit obtained with our sharpest (~ 20 ns) pulses is of the order ~ 100 fJ. However, the linear trend of V_T with nanogap size (Fig. 4B) reveals that such devices are highly scalable and suggests that ~ 5 -nm GST bits with CNT electrodes could operate at ~ 0.5 V and < 1 μ A, such that nanosecond switching times (29, 30) would lead to sub-femtojoule per bit energy consumption [for additional estimates see section 6 of (19)]. Low-voltage operation could also be achieved by using materials with lower threshold fields, such as GeSb (27). These results are encouraging for ultralow-power electronics and memory based on programmable PCM with nanoscale carbon interconnects.

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Low-Voltage, Low-Power, Organic Light-Emitting Transistors for Active Matrix Displays

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Intrinsic nonuniformity in the polycrystalline-silicon backplane transistors of active matrix organic light-emitting diode displays severely limits display size. Organic semiconductors might provide an alternative, but their mobility remains too low to be useful in the conventional thin-film transistor design. Here we demonstrate an organic channel light-emitting transistor operating at low voltage, with low power dissipation, and high aperture ratio, in the three primary colors. The high level of performance is enabled by a single-wall carbon nanotube network source electrode that permits integration of the drive transistor and the light emitter into an efficient single stacked device. The performance demonstrated is comparable to that of polycrystalline-silicon backplane transistor-driven display pixels.

Organic light-emitting diode (OLED) displays have well-recognized advantages in power consumption, pixel brightness, viewing angle, response time, and contrast ratio over liquid crystal displays (LCDs) (1). The primary technical challenge preventing wider commercial implementation remains the drive transistor in the active matrix (AM) backplane. Amorphous silicon (a-Si), the transistor channel material that

sources the voltage to switch AM-LCD pixels, has a low mobility (~ 1 cm² V⁻¹ s⁻¹) (2). To drive the currents necessary for OLEDs would require higher drive voltages, consuming power; moreover, its stability is unacceptable for AMOLED pixels (3, 4). For small AMOLED displays now in some handheld devices, the solution has been to use low-temperature polycrystalline silicon (poly-Si). However, poly-Si adds processing steps, time, and expense to the device fabrication (5, 6). Poly-Si also suffers from a more fundamental limitation: variation in the size, orientation, and number of the large polycrystalline grains (important to its high mobility for sourcing the high drive currents) leads to pixel-to-pixel inhomogeneity (7). This limits the production yield and

becomes an increasingly severe problem with increasing display size.

Organic semiconductor channel materials are attractive for their homogeneity, low cost, and the variety of means by which they can be deposited, but their best mobilities are similar to that of a-Si. In the typical thin-film transistor (TFT) architecture, low-mobility channel layers would require a large source-drain voltage to drive the necessary current. This consumes power in the transistor (as opposed to light production in the OLED), compromising the power savings. In one all-organic AMOLED demonstration, more power was dissipated in the drive transistor than in the OLED it was powering (8). Mitigating this by increasing the channel width of the drive transistor to source more current is not viable; to do so would reduce the fraction of pixel area available to the OLED, requiring a higher current density through the electroluminescent emitter to maintain the display brightness, reducing OLED lifetime (9). Alternatively, the low mobility of the organics could be compensated by making the channel length short, placing the source and drain terminals very close to each other; but that incurs the expense of high-resolution patterning.

We recently demonstrated a carbon nanotube enabled vertical field effect transistor (CN-VFET) that, intrinsic to its architecture, permits short channel lengths without high-resolution patterning and gave on-currents sufficient to drive OLED pixels at low operating voltages (10). Here, to realize the full benefit of the architecture, we integrate the OLED into the CN-VFET stack. We call such a device a carbon nanotube enabled vertical organic light-emitting transistor (CN-VOLET)

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(11). Here red, green, and blue CN-VOLETs are demonstrated that take full advantage of the low power dissipation of the integrated drive transistor and, further, exploit the high transmittance of the nanotube source electrode—greater than 98% across the visible spectrum—to yield a light-emitting transistor that emits light across its full aperture.

Vertical-type organic light-emitting transistors (OLETs) of varied designs and operating principles have appeared in recent years (12–18). The best performance was demonstrated by Nakamura *et al.* (18) in an architecture designated a metal insulator semiconductor (MIS)–OLET. Their Ir(ppy)₃ emitter-based OLET possessed an effective aperture ratio (19) of 45% and achieved a brightness of 500 cd/m² at gate and drain voltages of –30 V and –13.3 V, respectively. For a typical Ir(ppy)₃

OLED to emit at this luminous flux through an aperture transmitting 45% of its light, the OLED would need to emit at 1100 cd/m², which would in turn require a cathode-anode voltage of approximately –6.5 V (20). This implies that –6.8 V of the –13.3 V drain voltage used by the MIS–OLET was dropped across its integrated “transistor” portion, which then dissipated 51% of the total power.

Our CN-VOLET can be thought of as an OLED stack inserted between the organic semiconductor of a CN-VFET and its drain electrode with the latter made an electron-injecting, low-work function metal (Fig. 1) (10, 11). The CN-VFET substructure thus controls hole injection into the OLED layers. In the CN-VFET the nanotubes are spread as a dilute network, well above electrical percolation [image in fig. S1A and

transmittance spectrum in fig. S1B (21)] across the gate dielectric sitting on a bottom gate electrode. A metal electrode provides electrical contact along one edge of the network. Because the nanotubes are used as the source electrode, rather than as the active channel, no separation of metallic from semiconducting nanotubes is necessary. The organic channel layer is deposited as a thin film across the nanotubes and the exposed dielectric, and finally, in the case of the CN-VFET, a metal drain electrode is deposited onto the organic channel layer, completing the device. The transconductance originates from a gate field-induced modulation of the Schottky barrier height and width between the nanotubes and the organic channel layer (11). Insertion of the OLED layers between the CN-VFET channel layer and the drain integrates the driving transistor and the light emitter in the single, stacked device. Because of the strong gate coupling to the nanotube source electrode, the Schottky barrier modulation alone is sufficient to turn the devices off without the need for an overlying insulator as required by the MIS–OLET (18) and responsible for limiting its aperture ratio. Combined with the high optical transmittance of the nanotube source electrode and a transparent indium tin oxide (ITO) gate, our bottom emitting devices radiate light across their full aperture.

Figure 1 shows a schematic cross section of the CN-VOLET fabricated for each primary color. The material of each layer is listed in Table 1. The DNTT (22) organic channel layer, made thick to eliminate shorting paths from residual particulates in the carbon nanotube (CNT) source material, acquired a rough surface morphology (23) as indicated by the spikes in Fig. 1. Non-trivial planarization and strategic doping (24, 25) were used to overcome the issues arising from this roughness. Complete fabrication details including extensive experiments required to overcome the CN-VFET substructure roughness are provided in (21).

In operation, the CNT source electrode was held at ground potential, and the drain and gate were biased relative to ground. Figure 2 plots the performance characteristics for each color device. Current density and luminance versus voltage (*J*–*L*–*V*) output curves (Fig. 2, A to C) and transfer curves (Fig. 2, D to F) are shown. Each color CN-VOLET operates at a gate voltage (*V*_G) range of ±3 V; the luminance surpasses 500 cd/m² at drain voltages (*V*_D) of –6.8 V (red), –4.9 V (green), –5.7 V (blue), with contrast ratios (ratio of the luminance at a given *V*_D between the on and off states) approaching 10⁴. These voltages are well within the range of typical poly-Si–based AMOLEDs (26, 27). Negligible hysteresis was observed in the dual scan direction transfer curves (Fig. 2, D to F) (28). The gate leakage current density (*J*_G) remains below the drain current density (*J*_D) by one order of magnitude or more in the off state (*V*_G = +3 V) and by three orders of magnitude or more in the on state (*V*_G = –3 V). Optical micrographs for each color (Fig.

Fig. 1. Schematics of the red, green, and blue CN-VOLETs. The base of the device (i.e., the substrate, gate, source, and channel layers) is the same for each color device.

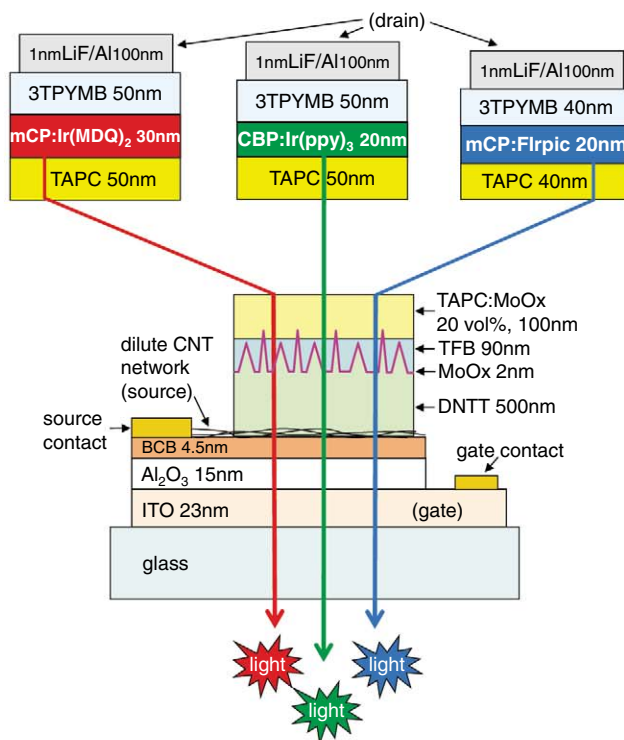


Table 1. CN-VOLET organic layers chemical name and function.

Abbreviation	Chemical name	Function
DNTT	Dinaphtho-[2,3-b:2',3'-f]thieno[3,2-b]-thiophene	High-mobility channel layer
TFB	Poly(9,9-dioctyl-fluorene-co-N-(4-butylphenyl)-diphenylamine)	Planarization channel layer
TAPC	1,1-Bis[(di-4-tolylamino)phenyl]cyclohexane	Hole transport layer
CBP	4,4'-N,N'-dicarbazole-biphenyl	Emitter host (green)
mCP	N,N'-Dicarbazolyl-3,5-benzene	Emitter host (red, blue)
Ir(MDQ) ₂	Iridium(III)bis(2-methylbenzo[f,h]quinoxaline)(acetylacetonate)	Red emitter
Ir(ppy) ₃	Fac-tris(2-phenylpyridinato)iridium(III)	Green emitter
Firpic	Bis[(4,6-di-fluorophenyl)-pyridinate-N,C2']picolinate	Blue emitter
3TPYMB	Tris[3-(3-pyridyl)-mesityl]borane	Electron transport layer

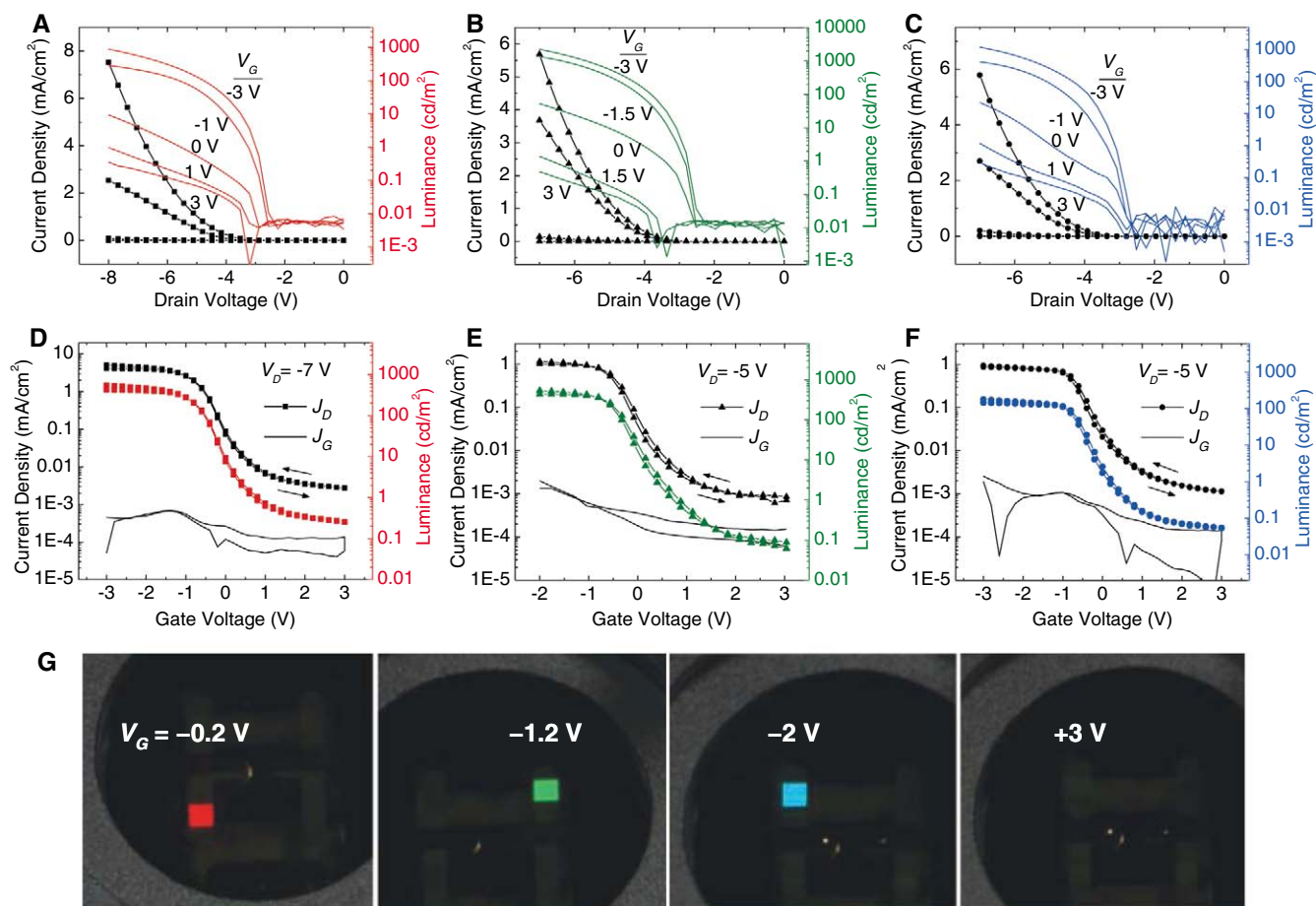


Fig. 2. CN-VOLET device operation. (A to C) Output and (D to F) transfer J - L - V curves of the (A and D) red, (B and E) green, and (C and F) blue CN-VOLETs. (G) Images of the corresponding color CN-VOLET pixels (1 mm by 1 mm) below the

graphs, at the indicated gate voltage. The diameter of the fixture opening is 14 mm. In the far right image, the blue CN-VOLET was in the fully off state (i.e., $V_G = +3$ V), which is also representative of the red and green CN-VOLETs in the off state.

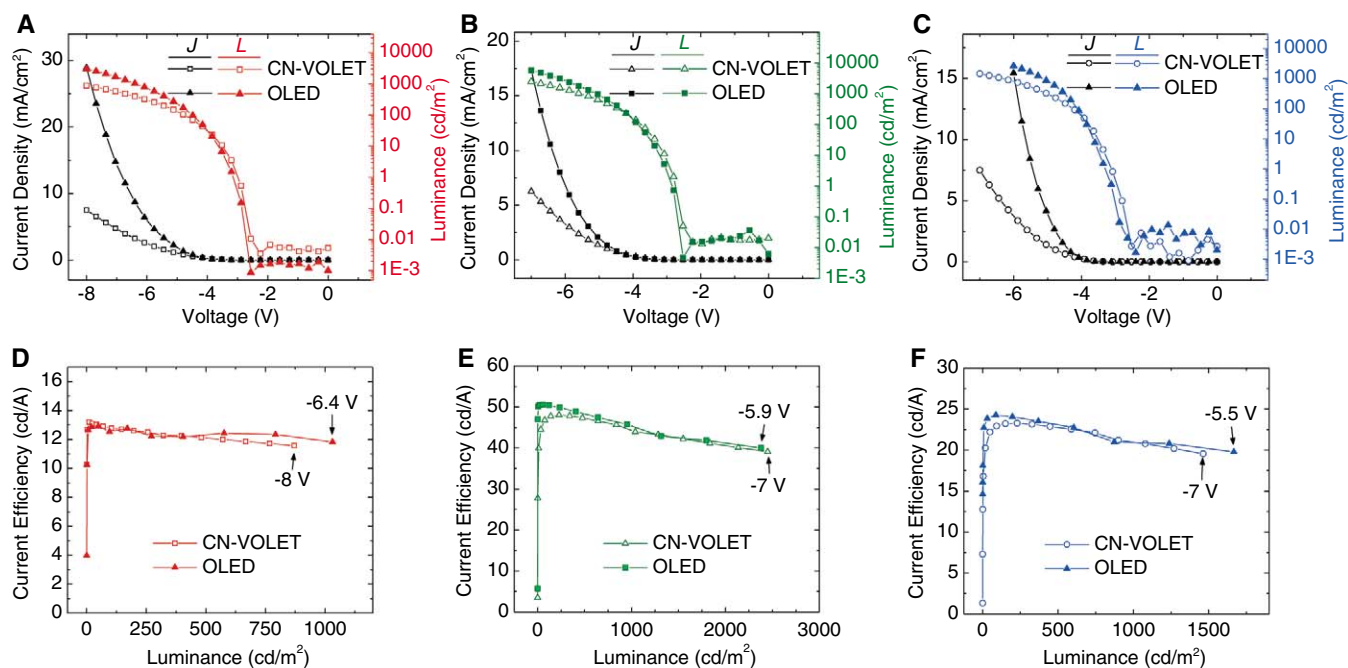


Fig. 3. Comparison of the CN-VOLETs to control OLEDs. J - L - V comparison for the (A) red, (B) green, and (C) blue CN-VOLETs in the on state (i.e., $V_G = -3$ V) to their respective OLEDs. Applied voltage is on the drain of the CN-VOLET (source grounded)

and on the cathode (anode grounded) of the OLED. Comparison of current efficiencies of (D) red, (E) green, and (F) blue CN-VOLETs ($V_G = -3$ V) to their respective OLEDs. Negligible light is absorbed and/or scattered in the base layers of the CN-VOLET.

Table 2. Turn-on voltages (29) and current efficiencies of red, green, and blue CN-VOLETs compared with their respective OLEDs.

Device		Red	Green	Blue
		Turn-on voltage (V)		
CN-VOLET		-2.57	-2.52	-2.71
OLED		-2.60	-2.53	-2.81
	Luminance (cd/m ²)	Current efficiency (cd/A)		
CN-VOLET		12.5	48.0	23.3
OLED	250	12.4	49.8	23.9
CN-VOLET		12.1	47.2	22.8
OLED	500	12.3	48.3	23.1

2G) show highly uniform light emission across the 1 mm by 1 mm pixels.

To determine the performance losses incurred by the integrated drive transistor in the CN-VOLETs relative to simple OLEDs, we fabricated control OLEDs on ultraviolet-ozone-treated ITO in the same evaporation runs that deposited the OLED layers on the CN-VOLETs. Figure 3 compares the OLED and CN-VOLET devices. In all cases, the CN-VOLET gate voltage was -3 V. The CN-VOLETs all turn on (29) at voltages slightly below that of their corresponding OLED (Table 2).

By comparing the current efficiency (i.e., the amount of light emitted per unit of current) for each CN-VOLET to that of its corresponding control OLED, we can quantify the decrease in light output incurred by the integrated transistor layers in each of the devices. The current efficiency for each CN-VOLET and its corresponding OLED are plotted as a function of luminance in Fig. 3, D to F, as the drain voltage for the CN-VOLET and the cathode voltage for the OLED were each swept from 0 V to the terminal value of the sweep indicated at the arrow. As is evident there, the integrated transistor layers have a negligible effect on the current efficiency. Table 2 compares the current efficiencies of the CN-VOLET and OLED at moderate and high brightness. Averaging these values indicates that, for equal luminance, the CN-VOLET emits light with 98% the current efficiency of the control OLED. Because the CN-VOLET emits light across its entire face, the effective aperture ratio (19) of the CN-VOLET can rationally be taken to be 98%.

The control OLEDs can also be used to determine the parasitic power consumption of the integrated transistor layers in the CN-VOLETs. At a display brightness of 500 cd/m², the transistor elements account for only 19, 6, and 15% of the total power consumption in the red, green, and blue pixels, respectively. Published organic drive technologies that also used the green phosphorescent Ir(ppy)₃ emitter allow for a direct comparison (Table 3). At 500 cd/m² display brightness (30), the side-by-side TFT+OLED and the MIS-OLET exhibit a parasitic power consumption greater than 50% against the CN-

Table 3. Comparison of effective aperture ratio and parasitic power dissipation percent between various OLED driving schemes.

Device*	Reference	Effective aperture ratio†	Parasitic power dissipation‡
TFT + OLED	(8)	50%	53%
MIS-OLET	(18)	45%	51%
CN-VOLET	This work	98%	6.2%

*All devices use the green phosphorescent emitter Ir(ppy)₃ lines (19). †Percentage of power dissipated across the driving transistor portion of the device not contributing to light generation; at 500 cd/m² display brightness (30).

‡This parameter excludes the switching TFT and addressing lines (19).

VOLET's ~6%—more than a factor of 8 difference. Currents in our pixel off state are higher than would occur for a conventional lateral channel TFT driving an adjacent OLED, but any power saving there would quickly be overtaken by the lower on-state efficiency of the latter. Moreover, the off-state power consumption of our pixels remains quite small. The average off-state current densities of our devices (Fig. 2) is 1.7 $\mu\text{A}/\text{cm}^2$. For a -5.7 V average drain voltage (giving a bright on-state average luminance of 350 cd/m²), this yields a power consumption for a 50-inch (127 cm) diagonal, 16:9 aspect ratio display, with every pixel in its off state, of 67 mW. For comparison, LCDs of this size consume 100 to 200 W, whether their pixels are on or off. Also listed in Table 3 are the effective aperture ratios (19) for the devices. The near-full aperture emission (~98%) of the CN-VOLET yields another major advantage: Because the integrated drive transistor of the CN-VOLET takes up no additional pixel area, the light emitter can occupy more of the pixel area, thereby achieving the same display brightness at substantially lower current density. This benefits OLED lifetime, which scales approximately as $1/J^2$, where J is the current density through the OLED (9).

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- The turn-on voltage is defined as the voltage at which the luminance surpasses 0.02 cd/m². This value is just above the off-state noise level measured by the Si photodiode used for the luminance measurements.
- The display brightness (L_D) accounts for the limited emitting area of the OLED as defined by its effective aperture ratio (A). To achieve a given display brightness, the OLED must be driven at a larger luminance (L_0). The display brightness can be defined as $L_D = L_0 \times A$.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/332/6029/570/DC1
Materials and Methods
Figs. S1 to S5
Table S1
References

19 January 2011; accepted 24 March 2011
10.1126/science.1203052

Proton-Catalyzed, Silane-Fueled Friedel-Crafts Coupling of Fluoroarenes

Oliver Allemann, Simon Duttwyler, Paola Romanato, Kim K. Baldridge, Jay S. Siegel*

The venerable Friedel-Crafts reaction appends alkyl or acyl groups to aromatic rings through alkyl or acyl cation equivalents typically generated by Lewis acids. We show that phenyl cation equivalents, generated from otherwise unreactive aryl fluorides, allow extension of the Friedel-Crafts reaction to intramolecular aryl couplings. The enabling feature of this reaction is the exchange of carbon-fluorine for silicon-fluorine bond enthalpies; the reaction is activated by an intermediate silyl cation. Catalytic quantities of protons or silyl cations paired with weakly coordinating carborane counterions initiate the reactions, after which proton transfer in the final aromatization step regenerates the active silyl cation species by protodesilylation of a quaternary silane. The methodology allows the high-yield formation of a range of tailored polycyclic aromatic hydrocarbons and graphene fragments.

Formation of carbon-carbon bonds via Friedel-Crafts methodology is one of synthetic organic chemistry's charter reactions, involving the attack by electron-rich π -clouds of aromatic rings onto carbocation-like substrates (1, 2). This reaction is of fundamental importance to industry (3), and its mechanistic principles also

pertain to many organometallic transformations (4). With respect to aryl-aryl couplings, Friedel-Crafts chemistry is limited by the thermodynamic instability of the phenyl cation (5); however, the importance of such couplings has mothered the invention of a plethora of noble and coinage metal-mediated methodologies (6, 7). These high-

ly useful aryl-aryl couplings typically involve an activated nucleophilic aryl partner, an aryl bromide or iodide, and a transition metal catalyst (8–10). Omission of the transition metal, selection of an aryl fluoride, and use of an unactivated aryl nucleophile are not contemplated by any reactive scheme currently in the chemist's repertoire (11, 12). Therefore, the efficient intramolecular coupling of an arene with an aryl fluoride by carbon-fluorine bond activation constitutes an avenue of synthetically useful Friedel-Crafts reactivity ripe for exploration.

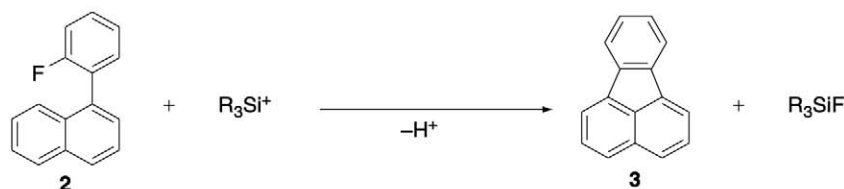
Carbon-fluorine bond activation by silyl cations has been applied to assist hydrodefluorination of benzyl and alkyl fluorides (13, 14). In addition, silyl cations are instrumental in supporting aliphatic carbon-carbon bond formation (15). The mechanism is ascribed to the formation of well-known alkyl cation equivalents, which can be trapped in classical Friedel-Crafts reactions. In contrast,

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Table 1. Conditions for C–F activation applied on 1-(2-fluorophenyl)naphthalene. Reactions were all conducted with 0.1 mmol of 1-(2-fluorophenyl)naphthalene under permanent inert atmosphere. The product

was purified by flash column chromatography. The conversion was measured by GC-MS; the isolated yields are reported only for conditions that led to complete conversion. Mes = 2,4,6-trimethylbenzene.



#	Cation	Anion	Base*	Solv.	T (°C)	Time (h)	Conv. (%)	Yield (%)
1	iPr_3Si^+	$[CB_{11}H_6Cl_6]^-$	$P(o-tol)_3$	PhMe	80	18	<10	–
2	iPr_3Si^+	$[CB_{11}H_6Cl_6]^-$	$P(o-tol)_3$	PhCl	110	15	100	80
3	iPr_3Si^+	$[CB_{11}H_6Cl_6]^-$	2,6-di- <i>t</i> Bu-pyridine	PhCl	110	8	100	90
4	Ph_3C^+/iPr_3SiH	$[B(C_6F_5)_4]^-$	$Me_2Si(Mes)_2$	PhCl	110	16	60	–
5	$iPr_3Si^+^\dagger$	$[CB_{11}H_6Cl_6]^-$	$Me_2Si(Mes)_2$	PhCl	110	8	100	93
6	$iPr_3Si^+^\ddagger$	$[CB_{11}H_6Cl_6]^-$	$Me_2Si(Mes)_2$	PhCl	110	17	100	90
7	$Ph_3C^+/iPr_3SiH^\dagger$	$[CB_{11}H_6Cl_6]^-$	$Me_2Si(Mes)_2$	PhCl	110	8	100	86
8	$Ph_3C^+/Me_2(Mes)SiH^\dagger$	$[CB_{11}H_6Cl_6]^-$	$Me_2Si(Mes)_2$	PhCl	25	15	<10	–
9	$Ph_3C^+/Me_2(Mes)SiH^\ddagger$	$[CB_{11}H_6Cl_6]^-$	$Me_2Si(Mes)_2$	PhCl	110	8	100	89
10	Mesitylene- $H^+^\ddagger$	$[CB_{11}H_6Cl_6]^-$	$Me_2Si(Mes)_2$	PhCl	110	8	100	92

*Entries 1–3 use an explicit base to neutralize the proton generated; entries 4–10 couple neutralization to the generation of the next silyl cation through a protodesilylation. † 10 mol-%. ‡ 4 mol-%.

aryl carbon-fluorine bonds appeared sufficiently inert in these studies such that aryl-fluorides could be used as solvents without interference; formation of phenyl cation was not anticipated (13, 14).

The driving force for the reaction comes from the substantial Si–F bond strength relative to that of the C(aliphatic)–F bond. Notably, the strength of the Si–F bond exceeds even that of the C(aryl)–F bond by about 120 kJ mol^{−1} (16, 17), and fluoride abstraction from fluoroarenes by silyl Lewis acids was recently demonstrated (18). Given these findings, a silylium-promoted Friedel-Crafts arylation appears feasible in terms of bond enthalpies and reaction kinetics.

In a brute-force approach, one would combine the organic substrates with a stoichiometric amount of a silyl cation to effect fluoride abstraction, and a Brønsted base to neutralize the Wheland intermediate (**B**, Fig. 1), which arises from attack by the nucleophilic arene on the incipient aryl cation. This strategy necessitates the availability of a full equivalent of a sufficiently reactive silylium-

like species. Indeed, addition of triisopropylsilyl carborane (**1**) (19, 20) to a substrate such as 1-(2-fluorophenyl)naphthalene (**2**) effects the activation of the carbon-fluorine bond and formation of a new arene-arene bond, leading to the product fluoranthene (**3**).

An equivalent of one proton is generated during the reaction; the neutralization of this charge by a sterically hindered Brønsted base prevents undesired polymerization reactions. A moment's reflection reveals that this proton need not be simply scavenged, but rather can be channeled into a productive part of the reaction. Protodesilylation of arylsilanes to yield an equivalent of a silylium ion and an arene is a well-known phenomenon relying on the greater bond enthalpies of C–H bonds over those of Si–C bonds (21–23). Given that the silyl cation-initiated Friedel-Crafts arylation formally generates one equivalent of HF, the proton formed after the first C(aryl)–C(aryl) coupling can serve as a catalyst to generate a silyl cationic species from a neutral silane precursor.

Overall, the exchange of covalent bonds [C(ar)–H, C(ar)–C(ar), and Si–F for C–(ar)–H, Si–C(ar), and C(ar)–F] is thermodynamically highly favorable. Thus, an elegant self-sustaining reaction system comprises the arene or aryl fluoride (as substrate) and an arylsilane (as fuel that consumes protons and releases silyl cations), plus a substoichiometric amount of a silyl cation (as initiator).

Substoichiometric use of the preformed silyl cation species described above only provides a shunt into this reaction manifold. The proton generated during the reaction (for example, as [fluoranthene-H]⁺[carborane][−]) is the actual catalyst. Arenium carboranes—protonated arenes paired with carborane counterions—are a young class of superacids that can be isolated in pure form (24). Thus, direct addition of an arenium carborane, instead of the silylium initiator, should effect a reaction wherein a proton serves as the catalyst to transform a neutral silane into an intermediate silyl cation. This cation then activates the carbon-fluorine bond, thereby inducing arene-arene coupling and release of a proton to recommence the process.

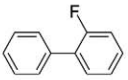
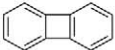
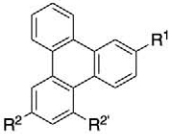
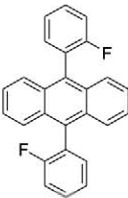
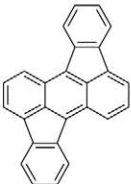
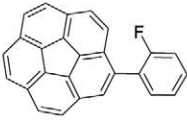
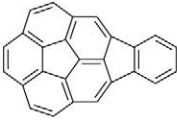
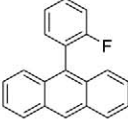
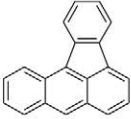
The substrate 1-(2-fluorophenyl)naphthalene (**2**) served as a model substrate for developing suitable Friedel-Crafts reaction conditions. Upon activation of the C–F bond, **2** undergoes ring closure to furnish fluoranthene (**3**). Initially, conditions involving stoichiometric amounts of silyl cation and a sterically hindered Brønsted base were investigated (25). Performing the reaction with *i*Pr₃Si–CB₁₁H₆Cl₆ and either P(*o*-tol)₃ or 2,6-di-*tert*-butylpyridine at 110°C for 8 to 15 hours led to high conversions and isolated yields of **3** (Table 1). Chlorobenzene proved to be a suitable solvent for this transformation and afforded better results than toluene. The more Lewis-basic toluene may deactivate the silylium carborane by π coordination (26). Intermolecular aryl-aryl coupling between toluene and the incipient phenyl cation was not observed.

The ring closure also proceeded effectively with substoichiometric silyl cation as initiator in combination with a stoichiometric amount of silane. This approach led to isolated yields of up to 93% using *i*Pr₃Si–CB₁₁H₆Cl₆ (**1**) and Me₂SiMes₂ (**4**). It is possible to carry out the reaction either with preformed or in situ generated cations (Table 1), the former giving slightly better yields. An attempt to run the reaction at room temperature yielded only small quantities of product.

Triggering the reaction with mesitylenium carborane (**5**) (a very strong Brønsted acid) resulted in complete consumption of starting material and high isolated yield of product. Triisopropylsilylium (**1**) and mesitylenium ion (**5**) act as initiators of the catalytic cycle (Fig. 1). They start the catalytic cycle at a different state (**B** and **C**) but eventually lead to the same active species, the mesityldimethylsilyl cation (**D**).

Counterions play a significant role in reactive cation chemistry; chemical stability and low nucleophilicity are preferred characteristics for clean cation reactivity. Two classes of anions,

Table 2. C–F activation reaction on various substrates; conditions from entry 5 of Table 1 were used for these transformations.

Educt		Product		Yield (%)
6		7		0*
8		9		97
10a	R ¹ =Me, R ² =H	11a	R ¹ =Me, R ² =R ³ =H	99
10b [‡]	R ¹ =H, R ² =Me	11a	R ¹ =R ² =H, R ³ =Me	99
12 [‡]	R ¹ =R ² =Me	11b	R ¹ =R ² =H, R ³ =Me	
		13a	R ¹ =R ² =Me, R ³ =H	98
		13b	R ¹ =R ² =Me, R ³ =H	
14		15		49
16		17		79
18		19		51 [†]

*Starting material recovered. [†]Partial product decomposition. [‡]Substrates **10b** and **12** form a mixture of products (**11a/11b** and **13a/13b**) in a ratio of 3:2 (a:b).

Fig. 1. Suggested catalytic cycle of the fluoride abstraction and the subsequent intramolecular attack, using the example of transformation of 1-(2-fluorophenyl)naphthalene to fluoranthene.

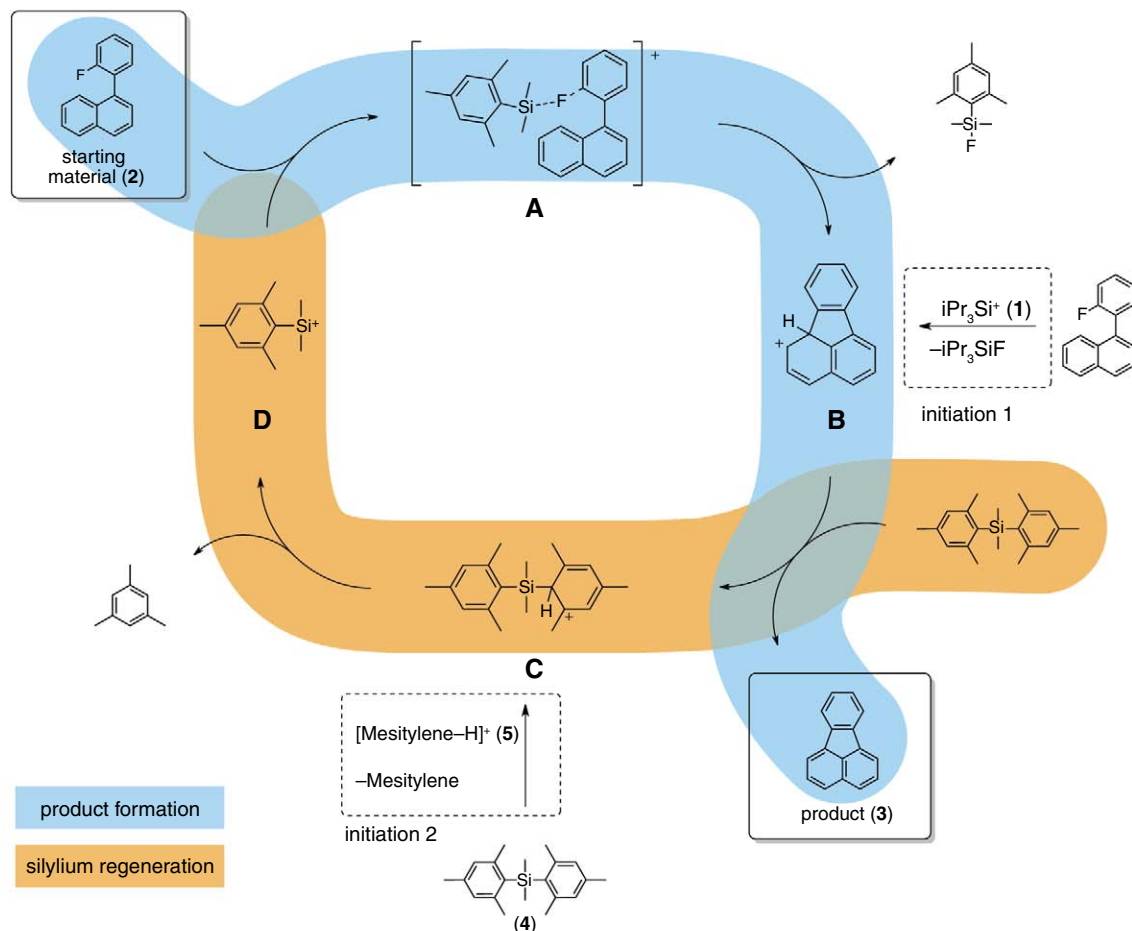
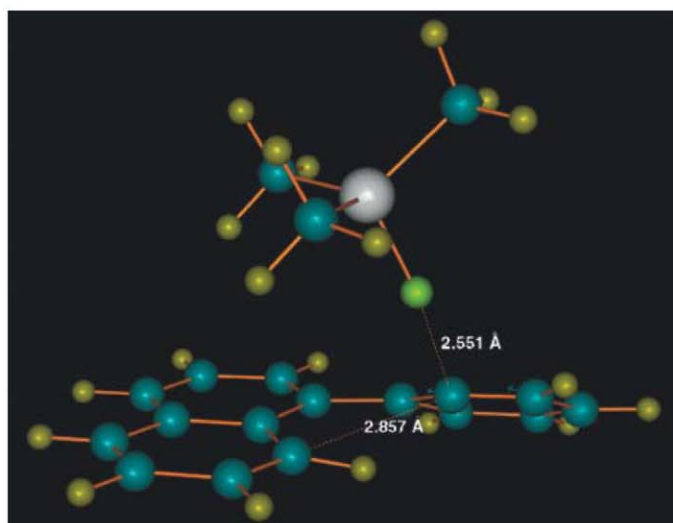


Fig. 2. MP2/DZ(2df,pd)//B98/DZ(2df,pd) calculated transition state (TS), including effects of chlorobenzene solvent, for fluoride abstraction from 1-(2-fluorophenyl)naphthalene (calculated $E_a = 19.8 \text{ kcal mol}^{-1}$; transition-state interatomic lengths, C–F = 2.55 Å, C–C = 2.86 Å).



perfluorinated tetraphenylborates and carboranes (27, 28), are very weakly coordinating anions with widely distributed negative charge (29). In the present case, the carborane $[\text{CHB}_{11}\text{H}_5\text{Cl}_6]^-$ afforded superior results to those with $[\text{B}(\text{C}_6\text{F}_5)_4]^-$, consistent with earlier reports showing a higher robustness of carboranes toward high temperature and Brønsted acidity relative to the perfluorinated phenylborates (24, 30). High acidity is an inherent

component of the acid-catalyzed reaction mechanism, and heating is necessary for the product to form within an adequate period of time.

It is likely that C–F activation does not take place by a completely dissociative mechanism, because $\text{C}_6\text{H}_4\text{R}^+$ would have a very unfavorable electronic configuration (31). Irrespective of the mechanistic details, after ring closure, the resultant protonated aryl moiety B is evidently acidic

enough to protonate the electron-rich mesityl ring of dimethyldimesitylsilane. Elimination of mesitylene reforms the silyl cation (D) and completes the catalytic cycle.

An early assessment of the scope of the reaction shows its applicability to the synthesis of a variety of polynuclear aromatic hydrocarbons (Table 2). It is effective in couplings involving fullerene fragments and could be an alternative to Scholl chemistry toward the formation of graphene and other carbon-based mesogens (32, 33). Reductive activation of carbon-halogen bonds, other than fluorine, has been used toward this goal of forming graphene subunits, but often steric crowding prohibits the formation of the necessary precursors. The small steric profile of the fluorine atom and the need to have only one derivatized partner adds substantial value to this methodology for preparing tailored graphene models.

For the formation of less strained five- or six-membered ring systems, conditions were adapted to obtain yields between 49 and 99%. In contrast, it has not yet been possible to generate the four-membered ring of biphenylene (7) from substrate 6 by applying this method. Terphenyl educts 8, 10a, 10b, and 12 showed full conversion to products 9, 11a, 11b, 13a, and 13b using optimized conditions (Table 1, entry 5). The yields were between 97 and 99%. Substrates 14 and 16 were more difficult to

transform to the desired products **15** and **17**. Additional silylium carborane and silane, plus extra hours of stirring, were required to achieve a higher yield. Treatment of substrate **18** afforded a yield of 51%; the lower yield was probably caused by decomposition of the product (**19**).

The terphenyl substrates containing a methyl group on the nonfluorinated phenyl ring (**10b** and **12**) showed a mixture of ortho and para products. The distribution was slightly in favor of the para position, which should be more accessible; however, the small preference for the para methyl indicates a very reactive intermediate that does not distinguish strongly between the two positions.

Additional studies enabled us to make a qualitative statement on the reaction rate. The terphenyl substrates (**8**, **10a**, **10b**, and **12**) were used in competitive C–F activation reactions. Two substrates were added to the same reaction mixture with only half an equivalent of reagent. Gas chromatography–mass spectrometry (GC–MS) consistently showed that substrates containing methyl groups (at any of the two rings) reacted faster than did the electron-poorer rings without substituents. The tendency of reaction rates for different substrates was found to be **8** > **10a** ≈ **10b** > **12** (**25**). This observation matches with the proposed transition state **A**. Its positive charge would be stabilized by an increased electron density of the fluorophenyl ring, provided by an additional substituent such as the methyl group. Methylation of the donor arene had the same effect on the reaction rate.

For the transformation of substrate **2**, quantum mechanical calculations predicted a transition state for the fluoride abstraction (Fig. 2) that nearby aryl moieties can stabilize (**18**, **25**). The structural and activation parameters predicted by the quantum chemical model (calculated $E_a = 19.8 \text{ kcal mol}^{-1}$; transition-state interatomic lengths, C–F = 2.55 Å, C–C = 2.86 Å) fit well with the experimental findings (**34**). The model further

supports the observation that a more electron-rich arene accelerates the reaction.

The proton is likely the universe's oldest catalyst, and it is now available as a crystalline arenium carborane (**25**). Coupled to a neutral silane as fuel, arenium acids are competent catalysts for the long-sought phenyl cation–based Friedel–Crafts reactivity. Such C–F activation for the formation of arene–arene bonds complements transition metal–based arene–arene couplings, particularly for the formation of designed graphenes and higher-order polynuclear aromatic hydrocarbon–based materials. Understanding the science of silyl cations and phenyl cation–like intermediates at play here, along with the design of tailored precursors, will certainly lead to a useful expansion of the synthetic chemist's tool box and the material chemist's objects of investigation.

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Supporting Online Material

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Table S1

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Venus's Southern Polar Vortex Reveals Precessing Circulation

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Initial images of Venus's south pole by the Venus Express mission have shown the presence of a bright, highly variable vortex, similar to that at the planet's north pole. Using high-resolution infrared measurements of polar winds from the Venus Express Visible and Infrared Thermal Imaging Spectrometer (VIRTIS) instrument, we show the vortex to have a constantly varying internal structure, with a center of rotation displaced from the geographic south pole by ~3 degrees of latitude and that drifts around the pole with a period of 5 to 10 Earth days. This is indicative of a nonsymmetric and varying precession of the polar atmospheric circulation with respect to the planetary axis.

Venus has the most extreme atmospheric circulation of the terrestrial planets, with the cloud-level atmosphere spinning on average 60 times faster than the planet's surface (**1**). This superrotation (**2–10**) extends to both

polar regions in hemispheric spiral-like patterns of clouds (**11**), where it results in fast rotating, infrared-bright central vortices (**12–15**).

Although the spiral hemispheric patterns can be seen in ultraviolet dayside imagery, which re-

veals upper cloud features at ~70 to 75 km altitude (**11**), thermal infrared observations reveal cloud structures at slightly lower altitudes of 65 to 70 km and show that the center of the southern polar vortex has a brightness temperature ~15 K warmer than a surrounding cold collar (**15**). The mean cloud-top altitude is close to 74 km up to mid-latitudes in both hemispheres but decreases

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Fig. 1. (A) The S-shaped pattern of the southern polar vortex, frequently referred to as the polar dipole. Indicated is the apparent inversion of the zonal wind between the pole (cross) and the true center of rotation (white circle) if the latter is displaced from the planetary axis. The gray arrow indicates a zonal (east-west) component that is locally opposite to the large-scale circulation (white arrow), showing that the centroid is displaced on average by 3° from the geographic south pole. The image is the $5.0\text{-}\mu\text{m}$ radiance map of the polar vortex from orbit 38 in latitude-local solar time coordinates. The outer circle is the 75°S parallel. **(B)** Complex morphologies seen in Venus's southern polar vortex during four Venus Express orbits. These radiance maps were obtained by the VIRTIS instrument at the wavelengths $3.8\text{ }\mu\text{m}$ [orbits 473 (i), 474 (ii) and 476 (iv)] and $5.0\text{ }\mu\text{m}$ [orbit 475 (iii)] (25). Orbit numbers represent days since orbit insertion at 11 April 2006.

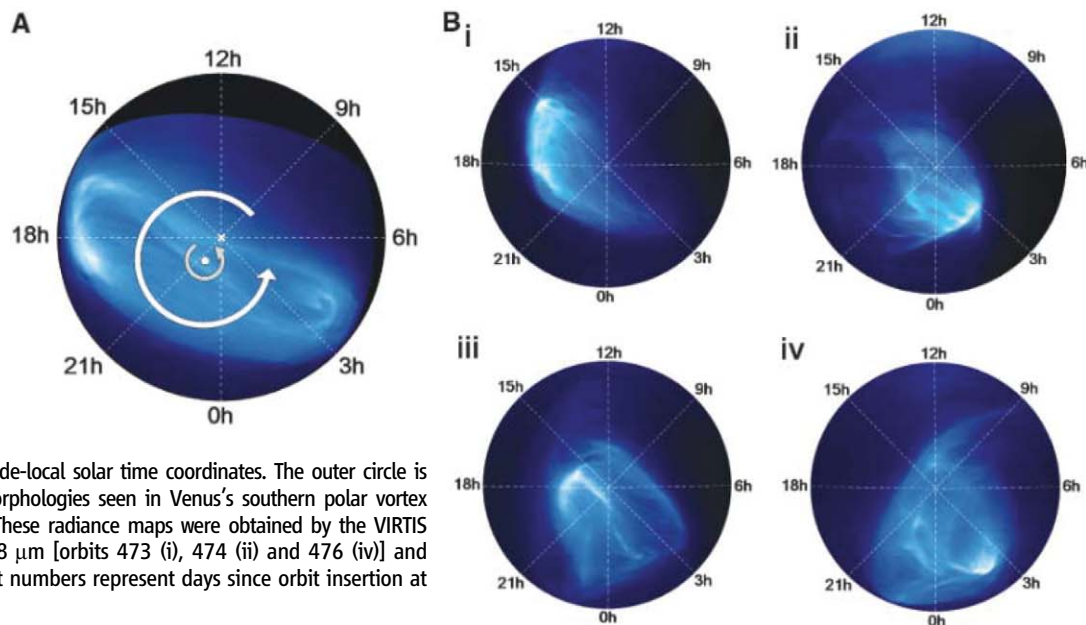


Fig. 2. Global mean (blue) zonal and (red) meridional wind components, obtained from feature tracking at $5.0\text{ }\mu\text{m}$. Negative zonal wind indicates retrograde (westward) rotation.

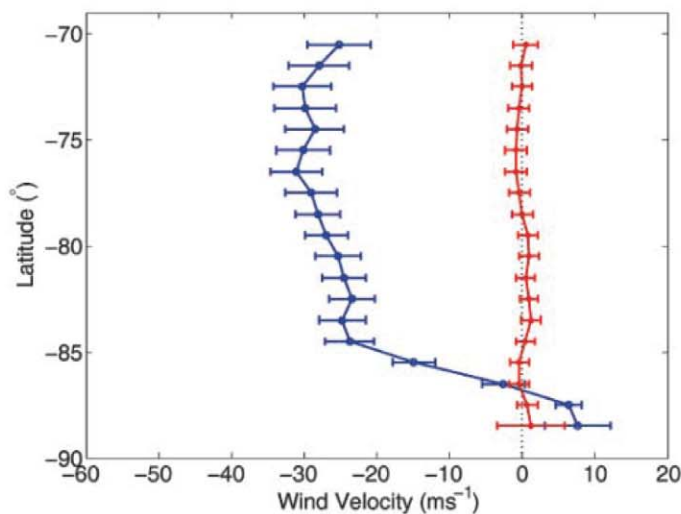
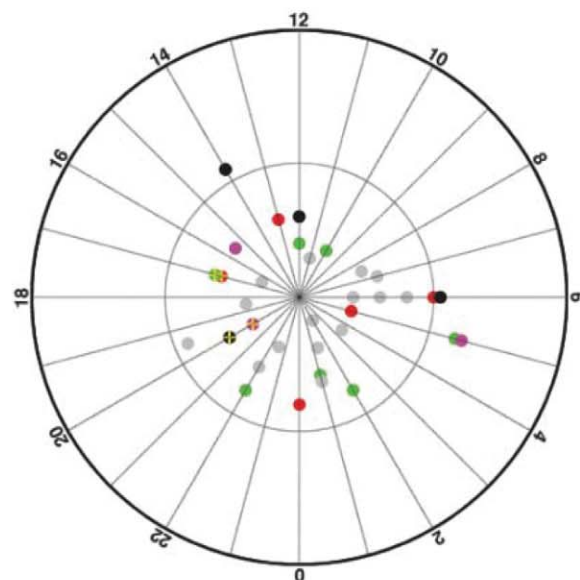


Fig. 3. Average positions of the centroids of the rotation in latitude-local solar time coordinates, as measured from observations of the polar region at 3.8 and $5.0\text{ }\mu\text{m}$, for a set of 34 orbits sampled from a period spanning 640 orbits. The colored points indicate sequential measurements made over short periods of up to 10 days (red, orbits 390, 392, 394, 396 and 398; green, orbits 473 to 479; black, orbits 668, 672, 674, and 678; and purple, orbits 640, 642, and 644); a cross indicates the first point in the sequence. The gray points indicate measurements that are not part of sufficiently long sequences. Superimposed points were displaced latitudinally by 0.25° for clarity. The outer circle is the 80°S parallel.



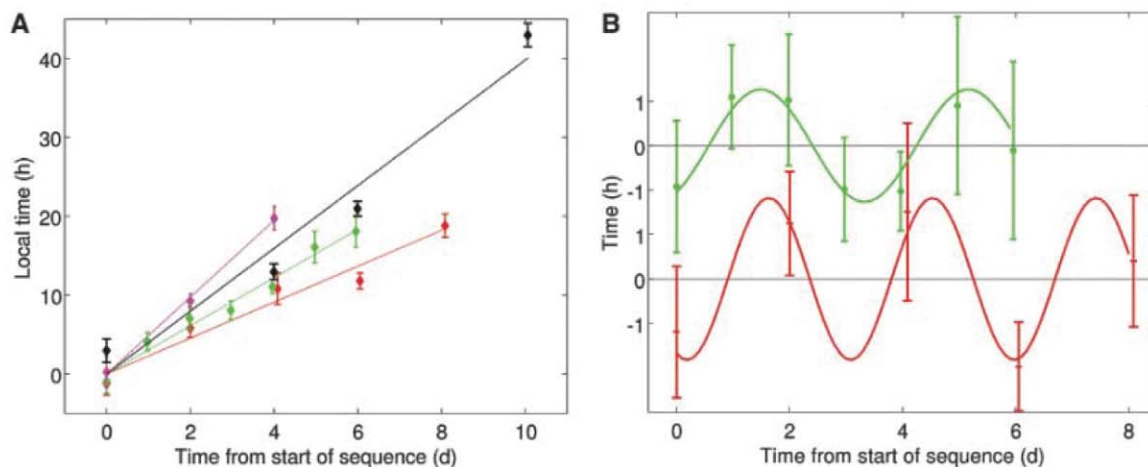
by 5 to 9 km at the pole and can experience fast variations (16). The decrease in cloud-top altitude, the increase in temperature, and the CO enrichment (17) at the center of the vortex may be indicative of downwelling in this region (12, 15).

The extended data set of Venus Express observations has shown the center of the southern polar vortex to be constantly changing in shape, on time scales of less than 24 hours. The longest imaging sequences obtained in a single orbit (~ 6 hours) generally show a rotating stable structure, but its morphology when imaged in the next orbit (24 hours later) bears little resemblance with that of the previous day (Fig. 1).

Previous studies of the dynamical behavior of the Venus polar vortices were limited by the spatial or temporal resolution of the data (14, 15), in particular by the resolution of the infrared bright structure at the center, known as the polar dipole. Rotation rates measured for the northern dipole at $11.5\text{ }\mu\text{m}$ (14) did not consider the fine structure of the vortex morphology, and the value obtained for its southern counterpart was based on a limited number of exposures. Although the northern polar vortex was occasionally seen to drift away from the pole (14), and this drifting was similarly observed once in its southern counterpart (15), this was not the subject of a detailed study.

Here, we present a study of the internal and global dynamics of the southern polar vortex from 70°S down to the south pole through use of high-spatial-resolution infrared images (at wavelengths of 3.8 and $5.0\text{ }\mu\text{m}$) from Venus Express's Visible and Infrared Thermal Imaging Spectrometer (VIRTIS) (18). A particular advantage of using these wavelengths for polar-feature tracking is that they can be used simultaneously in both day- and night-side areas of the planet, probing the cloud layer at ~ 65 km in the polar region (16).

Fig. 4. (A) Monotonic local time coordinate of the centroid of rotation and linear fit for a set of four series of orbits lasting between 4 and 10 days (same color code as in Fig. 3). We used sequences of three orbits or more; time is in days counted from the start of the series (26). **(B)** Residues of the linear fits for the two best sampled sequences.



The latitudinal wind profile in this upper cloud layer indicates a mean zonal wind that is retrograde and approximately uniform equatorward of 84°S , and a mean meridional wind that remains below the detection level (Fig. 2). The zonal component decreases strongly poleward of 84°S and changes sign to prograde close to 87°S . A slightly slower wind is detected at 75°S , coinciding with the poleward edge of the cold collar, which was previously identified as a region of colder air surrounding the bright center of the vortex (15). The inversion of the zonal wind close to the pole can be interpreted as a consequence of a nonalignment of the center of rotation of the infrared-bright feature with the planet's rotational axis. With such a displacement, an apparent inversion of the zonal wind between the pole and the true center of rotation results (Fig. 1A).

Clearly, the southern polar vortex is a morphologically variable, self-rotating, moving structure. It is also apparent that the position of its centroid of rotation is time variable over 24-hour periods and longer (Fig. 3). The majority of the centroids we measured are located poleward of 85°S , with a near uniform spatial distribution. The mean latitude is $87^{\circ}\text{S} \pm 1^{\circ}$, indicating that the center of polar vortex rotation is displaced from the south pole on average by 3° . These results, although admittedly from a small number of orbits, show no evidence of asymmetry of the positions of the centers of rotation with respect to local solar times, as might result from a solar tide-related forcing of the superrotation.

The centroid evolves around the pole in a precessional motion with near constant angular velocity (over time scales of up to a few days) (Fig. 4). Linear fits of the sequences yield motions of 2.3, 3.1, 4.0, and 4.9 hours/day in the local solar time frame (1 hour/day = 15° /day); the average motion is $\sim 52^{\circ}$ /day in the sense of the atmospheric rotation. This precessional motion is not uniform, however; it accelerates and decelerates (Fig. 4B). The residues of the linear fits can be fitted with sinusoids with periods of 2.5 days [orbits 390 to 398 (Fig. 4B, red)] and 3.6 days

[orbits 473 to 479 (Fig. 4B, green)], indicating an oscillation with a period close to 3 days.

Lastly, the morphological variation of the vortex center can be shown to be the result of differential rotation. We have computed the rotation rates about the centroid of rotation from the average zonal wind component obtained in each orbit. Mean rotation rates were computed for three different regions: the inner core within 5° of the centroid, the outer annulus also 5° wide, and the extended core (the inner core plus the annulus), 10° in radius. At cloud-top altitude, a 5° angular distance corresponds to 534 km along the meridians. Using this method, we measured periods of 2.3 ± 1.9 days for the inner core, 2.8 ± 1.9 days for the outer annulus, and 2.6 ± 1.9 days for the extended core region [compared with the rotation rate of 2.48 ± 0.05 days obtained previously (15)].

These results show that the southern polar atmospheric circulation is nonaligned with, and precesses about, the rotational axis of the planet. This may indicate that the region of maximum downwelling at the polar vortices—which is intimately linked with the general circulation of the atmosphere—is not static over the pole but drifts around it. In current theory, the Gierasch-Rossow-Williams mechanism for the origin of Venus's superrotation (19, 20) implies a poleward transport of momentum by the mean meridional circulation in the upper branch of the Hadley cells (which on Venus extend from the equator to the poles), balanced by equatorward transport by eddies in the mid-latitudes. Non-axisymmetric eddy motions are required because axially symmetric advective processes cannot account for the transfer of angular momentum from high to low latitudes (21). The nonalignment of the polar vortex with the planetary axis makes the polar circulation naturally non-axisymmetric and therefore capable of effecting this transport of momentum, as suggested previously (19). Moreover, because polar vortices are driven by the thermal contrast between the equator and the poles via the Hadley cells (22) the nonalignment and drift of the polar vortex may be an indication

that the region of subsidence in the descending branch of the Hadley cell is also nonaligned and drifting.

Polar vortices have interesting dynamical behavior, as has been shown in atmospheres throughout the solar system (23, 24). Our results highlight the importance of the polar vortex in the global dynamics of the Venus atmosphere, a phenomenon still not taken into account in current models of global circulation, which cannot simultaneously capture the dynamics of the vortices and superrotation.

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26. The local time coordinate, which is cyclical, has been linearized by adding 24 hours whenever a cycle was completed (for example, the sequence $[-5, 10, -1, -8]$ turns into $[-5, 10, 23, 40]$).

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Surface-Generated Mesoscale Eddies Transport Deep-Sea Products from Hydrothermal Vents

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Atmospheric forcing, which is known to have a strong influence on surface ocean dynamics and production, is typically not considered in studies of the deep sea. Our observations and models demonstrate an unexpected influence of surface-generated mesoscale eddies in the transport of hydrothermal vent efflux and of vent larvae away from the northern East Pacific Rise. Transport by these deep-reaching eddies provides a mechanism for spreading the hydrothermal chemical and heat flux into the deep-ocean interior and for dispersing propagules hundreds of kilometers between isolated and ephemeral communities. Because the eddies interacting with the East Pacific Rise are formed seasonally and are sensitive to phenomena such as El Niño, they have the potential to introduce seasonal to interannual atmospheric variations into the deep sea.

Hydrothermal vents are hot spots for geological, geochemical, and biological activity that alter the global oceanic heat and chemical budgets (1, 2) and support unique chemosynthetic communities (3, 4). The disjunct distribution and transient nature of vents pose challenges for exporting vent-derived heat and chemicals into the global ocean and transporting propagules between distant vent fields. On short time scales, vent-derived products can remain close to the ridge axis. Chemical tracers can be used to locate hydrothermal vents (5), and larval supply to established vents is predominantly from local sources (6, 7). However, larvae and much of the seawater chemically altered at vents are eventually transported to distant locales. Hydrothermal seawater alterations contribute to the removal and addition of major chemical constituents (1), such as Ca and Mg, as well as trace metals, such as Fe (8, 9) which may affect local and global biogeochemical cycling (10–12). Vent larvae must, at least episodically, undergo long-distance dispersal to maintain observations of

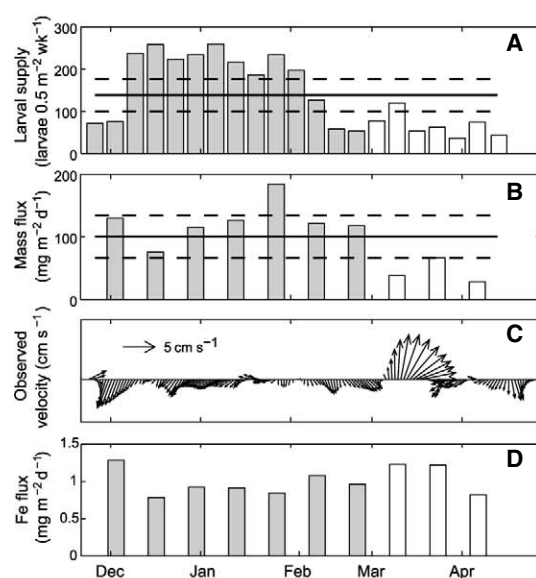
high gene flow (13, 14) and rapid colonization of disturbed and nascent vents (7, 15). Ridge-trapped jets (16, 17) and hydrothermally induced flows (18) have the potential to transport material near the ridge. Yet after decades of research at hydrothermal vents, the mechanisms by which heat, chemicals, and larvae are transported throughout the global ocean and between distant vent fields remain poorly resolved.

To investigate mechanisms that transport vent fluids and larvae, we performed time-series observations of hydrodynamics and larval, chemical, and mass fluxes in the 9°50' N area of the

East Pacific Rise (EPR) (fig. S1). Dramatic decreases in the larval supply of vent gastropods (Fig. 1A and figs. S2 and S3) and in mass flux associated with settling particles (Fig. 1B) were observed near the end of the time series and corresponded to a period of anomalous current velocities. Current velocities both on-axis (Fig. 1C and fig. S4, A and B) and off-axis (fig. S4C) reached speeds exceeding 15 cm s^{-1} during the anomaly, compared with the mean speed of 5.5 cm s^{-1} (at 170 m above bottom). Using a permutation test, larval and mass fluxes were significantly lower than expected post-anomaly (during and after the velocity anomaly): $P = 0.0007$ and $P = 0.008$, respectively. The biological and geochemical changes appear to have been driven by anomalous low-frequency currents (Fig. 1C) because the magnitude of high-frequency motions was relatively stable during this time (fig. S5).

The concurrent decreases (19) in larval supply and mass flux were most likely due to hydrodynamic transport away from the ridge rather than changes in source production. Both larval supply and mass flux are derived from independent pools built up over time, so changes in production would be observed as dampened or lagged changes in flux. Additionally, it is unlikely that mass flux sources and multiple species' reproduction changed concurrently. Hydrothermal vent gastropods typically exhibit continuous or quasi-continuous reproduction (20, 21) with pre-competency periods. Therefore, we assume our samples come from a continuously produced larval pool that integrates reproductive output over time.

Fig. 1. Observations at the EPR ridge crest, East Wall. (A) Supply of vent gastropod larvae and (B) mass flux. Solid line indicates the mean flux. Dashed lines indicate the 95% confidence interval (CI). (C) Current velocities recorded at 170 m above bottom (2350 m depth). Shown are the strong currents and rapid changes in direction in March 2005. (D) Variability of Fe flux over the time series. Solid bars indicate samples analyzed as before (pre-) the current anomaly. Open bars indicate samples during or after (post-) the current anomaly.



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In close proximity to vents that are distant from continents, mass flux is typically dominated by mineral particulates settling from the neutrally buoyant plume supplied by high-temperature “black-smoker” venting and the biogenic fluxes from adjacent chemosynthetic communities associated with low-temperature hydrothermal flow [supporting online material (SOM) text includes detailed geochemical analysis of the mass flux] (22, 23). Consistent temperatures and chemistry of vent fluids in November 2004 and April 2005 (24, 25) indicate that changes in high-temperature and low-temperature hydrothermal flow cannot account for the decreases in mass flux at the end of the time series. Instead, we suggest that the hydrodynamic event displaced the resident water

mass with its accumulated biogenic and mineral particulates, resulting in decreased mass flux. Although a reduction in mineral particulates from high-temperature venting [for example, Fe, Cu, and Zn (Fig. 1D and table S1)] was not observed during the hydrodynamic event, this does not necessarily imply that minerals and vent-altered seawater were not transported. Rapid production by vigorous venting, followed by prompt precipitation through quenching by cold, oxygenated bottom water, could replenish transported material over time scales that are short as compared with our sampling interval, thus masking any changes attributable to transport in the observed metal fluxes. Production of biogenic material from low-temperature vent communities would be mark-

edly slower so that displacement by the hydrodynamic event would be followed by delayed reestablishment of pre-anomaly fluxes. Thus, the observed variation in mass flux (Fig. 1B) and elemental fluxes (Fig. 1D and table S1) was probably due to transport of vent-derived mineral and biogenic material followed by swift replenishment of minerals by high-temperature venting but slower replenishment of the biogenic material.

The rapid changes in direction and coherence between current meters (Fig. 1C and fig. S4) separated by a few kilometers (fig. S1) are consistent with the inference that the hydrodynamic event observed on the ridge was due to the passage of a mesoscale feature. Daily objective analysis of satellite-observed sea-level anomalies revealed multiple eddies, including a ~375-km-diameter anticyclone originating in the Gulf of Tehuantepec in September 2004 that crossed the study site from February to March 2005 (Fig. 2 and movie S1), just before the anomalous bottom current velocities. Observed near-bottom current velocities were significantly negatively correlated with the inferred geostrophic flows associated with the eddy (Pearson correlation for the full 5-month record, $R_u = -0.20$, $P_u = 0.010$; $R_v = -0.21$, $P_v = 0.0073$). Given the potential for offset between the surface and deep flows and deformation and delay due to the ridge (26), we also allowed for a lag between the geostrophic (Fig. 2A) and near-bottom velocities (Fig. 1C), which improved the meridional correlation to $R_v = -0.57$ and $P_v < 0.0001$, with an 8-day lag. Although we cannot rule out the possibility of a jet on the ridge flanks sweeping across the site (16), the significant correlations between the observed deep currents and the surface geostrophic currents suggest a relationship between the anomalous current velocities at depth and the passage of the anticyclonic eddy.

Simulations with the 1/12° HYCOM ocean model (27) also showed intensified bottom current velocities associated with the passage of anticyclones at the end of the observational time series. As eddies formed off the coast of Central America and propagated westward, bottom-current velocities developed from weak, incoherent flows at the beginning of 2005 into coherent features with increased velocities by February (Fig. 3 and movie S2) and extending through the end of the time series. Simulated currents near the ridge and at the surface (fig. S6) were significantly correlated over 90 days ($R_u = 0.33$, $P_u = 0.0015$; $R_v = -0.54$, $P_v < 0.0001$). Including a lag further improved the meridional correlation [$R_v = -0.62$, $P_v < 0.0001$ (lagged 5 days)]. The strength of the cross-correlations and lag for the simulated currents are roughly consistent with those for our observations. Cross-correlations between near-bottom observed and simulated velocities from January through March 2005 were significant for the meridional component ($R_v = 0.51$, $P_v < 0.0001$) (fig. S6) but not for the zonal component ($R_u = 0.16$, $P_u = 0.14$). Eddy-associated meridional flows may have been intensified along the north-south trending

Fig. 2. Surface and deep expression of a mesoscale eddy. (A) Inferred geostrophic velocities at the surface and the associated time series of sea-level anomalies (SLAs) from November 2004 to May 2005. Two eddies pass during this time period: one in February to March during the period of bottom observations and a second from the end of April to May, just after the period of near-bottom observations. (B) Map of SLA on 21 February 2005 when an anticyclonic eddy made impact with the study site (white star). The track of the center of the eddy from birth on 10 September 2004 until exit from the study region on 1 May 2005, marked every 4 weeks (black circles), is superimposed on the map.

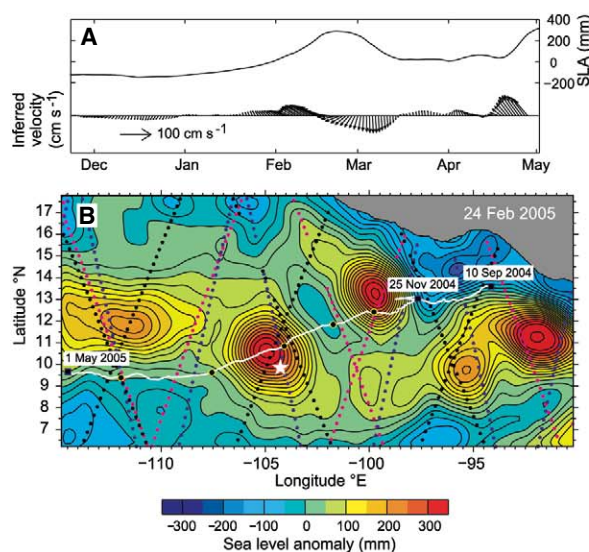
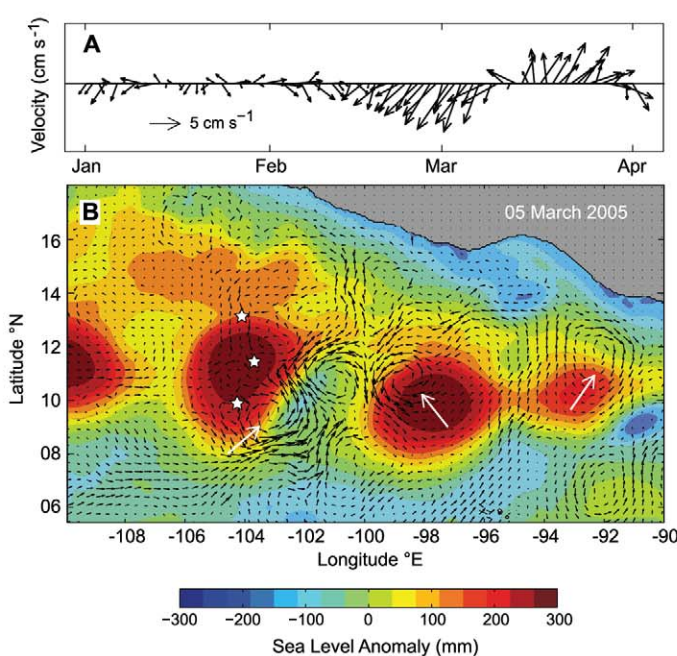


Fig. 3. HYCOM model simulation of anticyclone-induced current velocities at depth. (A) Simulated bottom currents at East Wall at 2350 m depth from January to April 2005. (B) Simulated sea-surface height variations for 5 March 2005 (color shading) consistent with assimilated satellite observations of a Tehuantepec eddy over hydrothermal vent fields (white stars), including our study site (southern-most white star). The model also predicts intensified and coherent near-bottom currents (black arrows) associated with the eddy field. For clarity, every fourth vector is plotted. The induced near-bottom currents are complex but often include deep cyclones associated with anticyclones (indicated by white arrows) consistent with the significant negative lagged correlations between the inferred geostrophic velocities and observed near-bottom velocities.



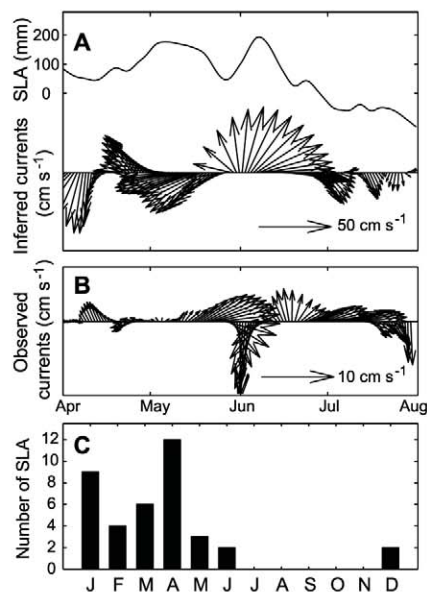


Fig. 4. Multiple eddies interact with the EPR at depth. **(A)** SLAs and inferred geostrophic currents show the passage of two anticyclonic eddies over the 9–10°N vent field from May through June 2007. **(B)** Coincident with the SLA, near-bottom current anomalies in the opposite direction were recorded on the EPR at 9°50.0' N, 104°17.4' W at 2430 m depth. Plots of SLAs and inferred currents were lagged 8 days relative to the observed near-bottom current velocities. **(C)** Mesoscale eddies cross the EPR seasonally. Analysis of satellite altimetry from January 1993 to December 2009 indicates that an average of 2.2 ± 0.9 (SD) eddies crossed the EPR each year, predominantly during the first half of the year.

ridge through topographically induced, meridional eddy elongation (26) or topographically rectified flows (16), whereas the zonal flows may have diminished into the background flows during deformation by the ridge axis or been influenced by fine-scale topography not included in the model, resulting in lower correlations. Still, these comparisons suggest that eddy-induced current velocities at depth were present during the period of observed current velocity anomalies and associated decreases in mass and larval fluxes.

Our findings suggest that eddy-driven impacts extend beyond the upper ocean and main thermocline (28) to include deep-sea benthic environments. Eddies at depth could play a major role in transporting hydrothermal vent-derived heat, chemicals, and biota in a relatively low-energy environment. Transport could occur wherever mesoscale eddies interact with ridges—including the Mid-Atlantic Ridge, the Southwest Indian Ridge, and the East Scotia Ridge (29, 30)—and the surrounding deep ocean. The observed strong decreases in larval and mass fluxes, together with a change in chemical compositions of the settling flux, during and after the current anomaly suggest that the passage of the eddy over the study site removed vent-altered seawater, larvae, and

associated biogenic matter. The observed local removal of larvae would be followed by inevitable loss to unsuitable habitat but could also result in the delivery of those larvae to distant vent fields. Complex interactions within the simulated eddy field created an intensified deep northward current that extended nearly 8° of latitude and interacted with the ridge for weeks (Fig. 3B and movie S2), potentially providing a conduit for larval transport between vent fields. The deep-water expression of large and/or interacting eddies could thus disrupt more persistent pathways of larval supply, such as local retention (6, 7) and transport by rectified mean flows (16, 17). However, the eddies provide a hydrodynamic mechanism to facilitate long-distance dispersal events, such as that of *Ctenopelta porifera* observed at nascent vents over 300 km away from the nearest known source (7). A few long-distance dispersal events driven by these eddies could produce high gene flow among vent fields and, more broadly, in the deep sea in general.

There is the potential for multiple eddies to interact with the EPR each year (31–33). A second instance of a strong negative correlation ($R_u = -0.56$, $P_u < 0.0001$; $R_v = -0.86$, $P_v < 0.0001$ with an 8-day lag) between a current anomaly observed on the ridge crest and inferred geostrophic velocities at the surface was detected from May through June 2007 (Fig. 4, A and B), lending further support for eddy-induced current velocities at depth. In the eastern Pacific, an average of 3.5 ± 1.2 (SD) Tehuantepec and 2.2 ± 1.0 (SD) Papagayo eddies (31) form each year during the late fall to early spring (31–33); a subset of these eddies, 2.2 ± 0.9 (SD) per year, cross the EPR during the winter and spring (Fig. 4C). In addition to the seasonal production of these eddies, Tehuantepec eddies strengthen and increase in frequency during El Niño years (31–33). Although the deep sea and hydrothermal vents in particular are often naively thought of as being isolated from the surface ocean and atmosphere, the interaction of surface-generated eddies with the deep sea offers a conduit for seasonality and longer-period atmospheric phenomena to influence the “seasonless” deep sea. Thus, although hydrothermal sources of heat, chemical, and larval fluxes do not exhibit seasonality, there is potential for long-distance transport and dispersal to have seasonal to interannual variability.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/332/6029/580/DC1
Materials and Methods

SOM Text
Figs. S1 to S6
Table S1
References
Movies S1 and S2

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Brain Evolution Triggers Increased Diversification of Electric Fishes

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Luke J. Harmon,² Matthew E. Arnegard³

Communication can contribute to the evolution of biodiversity by promoting speciation and reinforcing reproductive isolation between existing species. The evolution of species-specific signals depends on the ability of individuals to detect signal variation, which in turn relies on the capability of the brain to process signal information. Here, we show that evolutionary change in a region of the brain devoted to the analysis of communication signals in mormyrid electric fishes improved detection of subtle signal variation and resulted in enhanced rates of signal evolution and species diversification. These results show that neural innovations can drive the diversification of signals and promote speciation.

Although we assume that sensory processing is fundamentally important for the detection of species-specific communications, or “signals” (1), we know relatively little about how brain evolution might affect signal divergence and speciation. African electric fishes within the family Mormyridae provide an ideal model system for relating brain evolution to diversification. The >200 described species in this family are phylogenetically and phenotypically diverse (2–8); communicate using brief, species-specific, and easily quantified electric signals (9); and process these signals in a well-defined sensory pathway devoted solely to the analysis of electric communication signals (10–12) (fig. S1).

Mormyrids generate electric signals to communicate and to actively sense their environment (11). These signals have evolved more rapidly than body shape, size, and trophic ecology, suggesting that electric communication behavior has played a key role in the radiation of mormyrids (3). Further, playback experiments in a few species suggest that these signals are critical for species recognition during mate choice (13–16). Electric signals are generated by an electric organ in the tail, which consists of electrically excitable cells called electrocytes (17). Electrocyte stalks evolved with the origin of mormyrids, and developmental flexibility in stalk morphology arose with the origin of the subfamily Mormyriinae. This evolutionary change in the Mormyriinae es-

tablished enhanced capacity for signal variation that is lacking in the Petrocephalinae, the only other mormyrid subfamily (Fig. 1).

Mormyrids have three types of electroreceptors: ampullary organs, mormyromasts, and

knollenorgans (18). Communication behavior is mediated exclusively by knollenorgans (10). In a region of the midbrain called the extero-lateral nucleus (EL; fig. S1), the timing of responses of knollenorgans located on different parts of the body is compared to extract information about electric signals (10–13). Despite the importance of the EL for signal analysis, EL anatomy has only been characterized in a few species (10–12). To investigate the role of brain evolution in mormyrid diversification, we performed a comparative analysis of EL anatomy. We obtained serial sections from the brains of 26 species (table S2). After standard histological processing, we delineated the borders of the EL in each section using established criteria (12). We then calculated total EL volume normalized to brain mass [see supporting online material (SOM)].

Previous studies identified distinct anterior and posterior subdivisions in the EL, referred to as ELa and ELp, respectively (10). Sixteen of the species that we studied clearly have separate ELa/ELp subdivisions (Fig. 2A). However, the

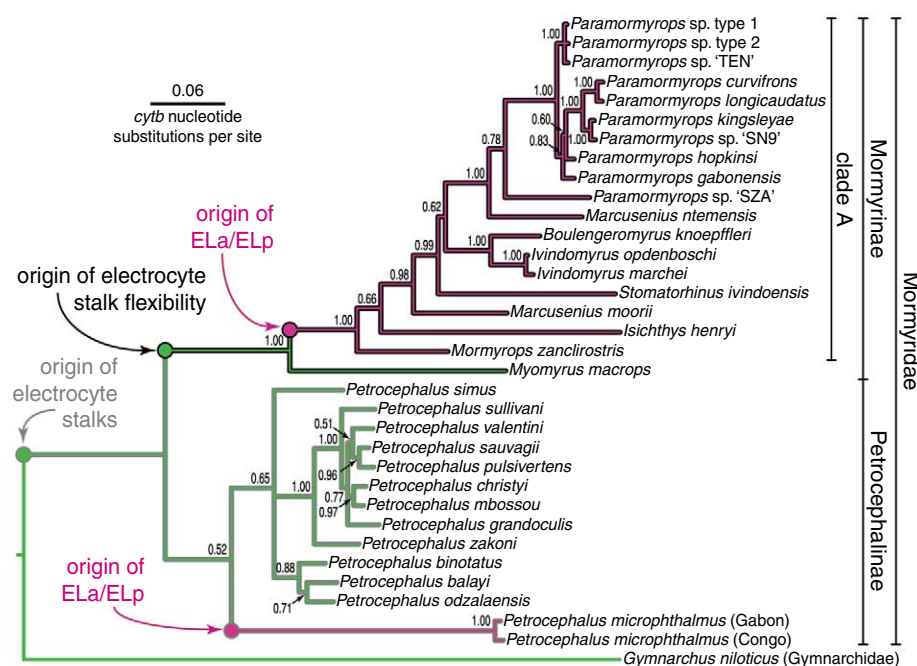


Fig. 1. Inferred tree of phylogenetic relationships among mormyrid species and morphs. The phylogeny was estimated by Bayesian analysis of *cytb* sequences (values at nodes are posterior probabilities). A sequence from the closest outgroup to the Mormyridae (*Gymnarchus niloticus*) was used to root the tree. Green branches represent a small extero-lateral nucleus (EL) and magenta branches represent an enlarged EL divided into anterior and posterior subdivisions (ELa/ELp); we reconstructed ancestral states using parsimony (see text). Gray outline represents electric organs with electrocyte stalks, and black outline represents electric organs with developmentally labile stalks, based on a previous study (2).

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remaining 10 species have a relatively small EL without any apparent subdivisions (Fig. 2B). Within the subfamily Mormyriinae, the genus *Myomyrus* has a small EL, whereas all other genera have an enlarged and subdivided ELa/ELp (Fig. 2C and table S2). The monophyly of the latter group is strongly supported by our *cytb* phylogeny (Fig. 1), as well as several published studies that used multiple molecular markers (2–6). We therefore refer to this lineage as “clade A,” and we conclude that all species in this lineage have an ELa/ELp (Fig. 1). Within the subfamily Petrocephalinae, we find that *Petrocephalus microphthalmus* is the only species with an enlarged ELa/ELp (Fig. 2C and table S2).

This pattern of EL anatomy suggests two equally parsimonious scenarios: Either the EL was ancestral and an enlarged ELa/ELp evolved twice independently, or ELa/ELp was ancestral, and a reduced EL evolved twice independently. To distinguish between these two possibilities, we related EL anatomy in mormyrids to a published description of midbrain anatomy in the monotypic Gymnarchidae, *Gymnarchus niloticus* (19), the sister taxon to all mormyrids (Fig. 1). This study identified an EL homolog without subdivisions, leading us to conclude that the EL is the ancestral mormyrid character state and that an enlarged ELa/ELp evolved twice, once at the origin of clade A and once in *P. microphthalmus* (Fig. 1).

Previous work has identified two distinct patterns of knollenorgan receptor organization:

a broad distribution of receptors throughout the body, or discrete clusters of receptors on the head (20). We hypothesized that this difference might relate to variation in EL anatomy. To test this hypothesis, we mapped knollenorgan locations in 15 species (see SOM) and combined our findings with published descriptions (6, 20, 21), yielding data on 11 clade A species, 2 *Myomyrus* species, and 13 petrocephaline species (table S2). All clade A species and *P. microphthalmus* have broad knollenorgan distributions (Fig. 3, A and B, and fig. S2B). With one exception (*Petrocephalus zakoni*), all other petrocephaline species have clusters of knollenorgans located only on the head (Fig. 3C and fig. S2C). *Myomyrus* has an intermediate phenotype, with a single cluster on the head as well as a low-density, broad distribution (Fig. 3D and fig. S2C). Thus, despite extensive interspecific variation, ELa/ELp is universally associated with a broad distribution of knollenorgans (table S2).

Mormyrids analyze electric signals in the EL by comparing the response times of knollenorgans located on different parts of the body (10–13), suggesting that species with broad receptor distributions should be better equipped for signal discrimination. Therefore, we hypothesized that ELa/ELp evolved to facilitate the processing of receptor responses for signal analysis, which predicts that species with an ELa/ELp should be better at detecting signal differences than species with an EL. To test our hypothesis, we performed

playback experiments in the field (see SOM). Previous research identified two distinct behavioral responses to electrosensory stimulation: increases in electric discharge rate (22), or pauses in electric output (23). We observed both responses. Among the six clade A species tested, four responded to stimulation with rate increases, and one responded with pauses. In the one remaining species (*Brienomyrus brachyistius*), two individuals responded with rate increases, and two responded with pauses. All four petrocephaline species responded with pauses.

In every species, repeated presentation of the same stimulus led to a decrease in response (i.e., habituation; fig. S4). We therefore used a habituation-dishabituation paradigm to assess signal discrimination ability in the field. Both control and experimental stimuli consisted of 10 bursts of 10 pulses each. For controls, all 100 pulses were an identical conspecific signal. Experimental stimuli were the same except that all 10 pulses in the ninth burst were a phase-shifted version of the same signal (fig. S3). Signal

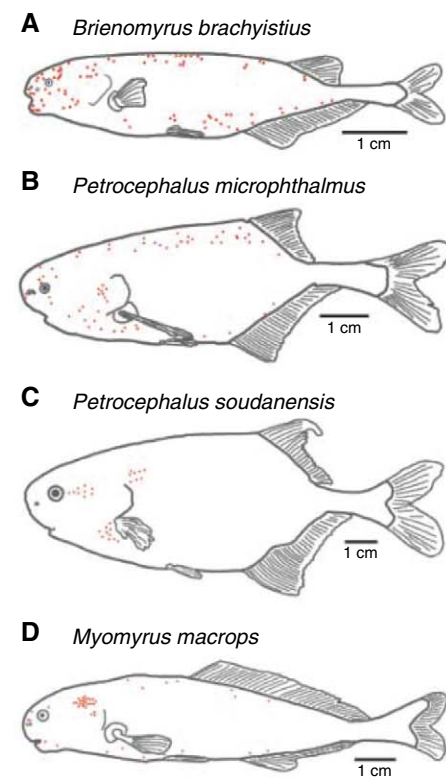
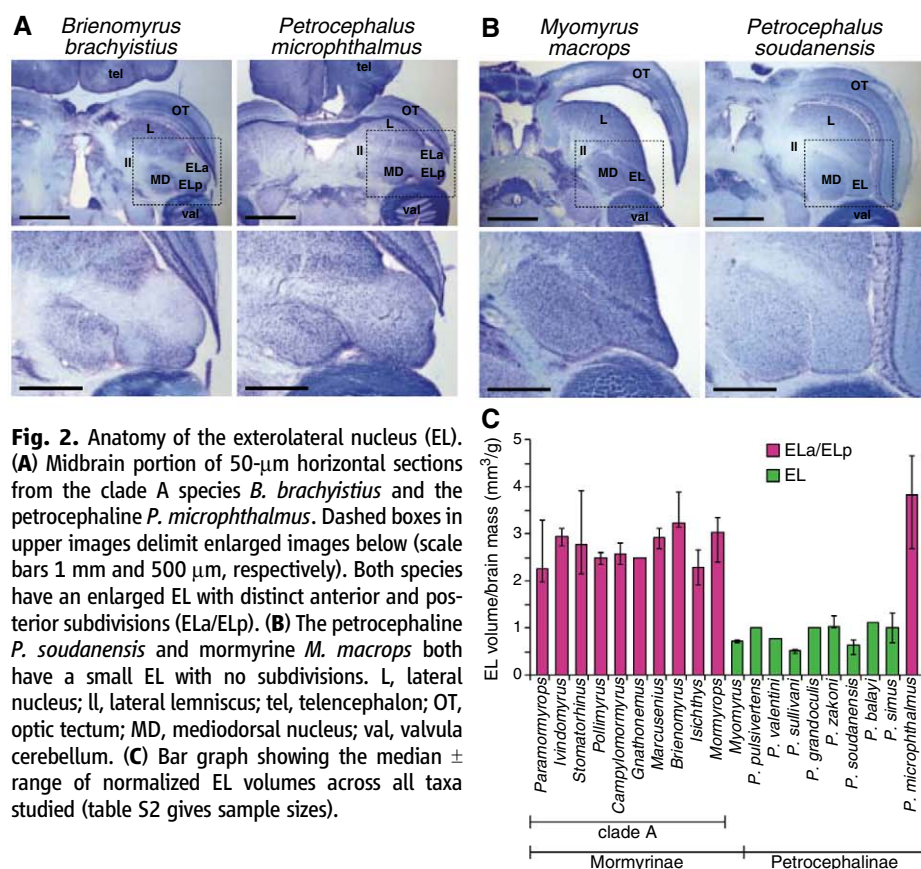


Fig. 3. An enlarged and subdivided exterolateral nucleus (ELa/ELp) is universally associated with broadly distributed knollenorgan electroreceptors. Knollenorgan locations are indicated by red dots. (A) *B. brachyistius* has a broad distribution of knollenorgans, as found in all clade A species. (B) *P. microphthalmus*, the sole petrocephaline species with an ELa/ELp, also has a broad knollenorgan distribution. (C) *P. soudanensis* has three knollenorgan clusters. (D) *M. macrops* has an intermediate pattern, with a single cluster and a low density of knollenorgans throughout the body.

discrimination was assessed as the change in response from the eighth to ninth burst. In all clade A species, inserting a phase-shifted signal into the stimulus train led to a partial recovery of response (i.e., dishabituation) (Fig. 4A; discharge rate increases: $n = 34$, $z = 4.57$, $P < 0.00001$; pauses: $n = 7$, $z = 2.37$, $P < 0.05$). The three petrocephaline species with an EL showed no evidence of discrimination (Fig. 4A; $n = 12$, $z = 1.49$, $P = 0.13$). However, in *P. microphthalmus*, the sole petrocephaline species with an ELa/ELp, the phase-shifted signal elicited dishabituation (Fig. 4A; $n = 10$, $z = 2.80$, $P < 0.01$). These results demonstrate that the independent evolution of ELa/ELp established signal discrimination abilities that are lacking in species with an EL.

Animal communication depends on both senders and receivers; in addition to the sensory capacity for signal discrimination, traits permitting the evolution of signal variation are necessary for a communication system that promotes diversification. Therefore, we hypothesized that rapid signal divergence and species diversification should be restricted to clade A, the only mormyrid lineage with both ELa/ELp and developmentally flexible electrocytes (Fig. 1).

To test the importance of these traits on rates of signal divergence, we analyzed the electric signals of species collected in two locales: the Ivindo River of Gabon (3, 9) and Odzala National Park of the Republic of the Congo (5, 6), homes to the largest known assemblages of Mormyriinae and Petrocephalinae, respectively. The combined data set represents 18 species and morphs within clade A and 13 outgroup species (fig. S5). Using cross-correlation (fig. S6) and multidimensional scaling (see SOM), we computed coordinates of all 407 signals in this data set within a two-dimensional space (Fig. 4B). We then computed squared Mahalanobis distances (D^2) between the centroids of each species or morph and plotted these values against ultrametric phylogenetic distances based on our phylogeny (Fig. 1). Assuming no particular model of evolution, the resulting plot reveals greater signal variation and more rapid signal divergence in clade A (Fig. 4C). We then formally compared signal divergence rates using a Brownian motion framework (24). Considering dimension 1, which reflects variation in temporal features of the signals (Fig. 4B), we found that the rate of signal divergence in clade A is more than 10 times fast-

er compared to that of other mormyrids ($\sigma^2_A = 0.000417$, $\sigma^2_{\text{other}} = 0.000037$, $\text{AICc} = 13.9804$, $P < 0.0002$). For dimension 2, which reflects other signal features, we found a twofold higher rate of signal divergence in clade A, but the difference was not significant ($\sigma^2_A = 0.000224$, $\sigma^2_{\text{other}} = 0.000118$, $\text{AICc} = -1.1484$; $P = 0.25$).

To test our hypothesis that clade A has experienced higher rates of species diversification, we compared diversification rates in clade A to closely related outgroup lineages (25). There are at least 175 extant species in clade A (7). By contrast, there are only 3 known species of *Myomorphus*, and only 30 known species in the entire subfamily Petrocephalinae (5–7). Every family of Osteoglossomorpha outside the Mormyridae contains 10 or fewer extant species (7). Moreover, the only two known osteoglossomorph species flocks are restricted to clade A (3, 5, 8). We find statistical support for net rates of diversification in clade A that are three to five times higher than those in closely related outgroup lineages (see SOM). This result is robust across a range of possible average extinction levels (table S3), supporting our hypothesis that the evolution of ELa/ELp triggered explosive diversification in clade A compared to that of other mormyrid lineages (Fig. 1). However, this same evolutionary change in *P. microphthalmus* did not trigger rapid diversification. We propose that the lack of developmentally flexible electrocyte stalks within the Petrocephalinae (2) impeded rapid diversification through signal divergence in the *P. microphthalmus* lineage.

Evolution of communication systems can have profound effects on species radiation. Evolutionary change in the structure of the anuran inner ear may have fostered increased rates of speciation through its effects on vocal communication (26). Similarly, the adaptation of visual receptors to different light environments has contributed to cichlid speciation through its effects on mate recognition (27, 28). The resulting radiations of species can then lead to evolutionary divergence in brain structure as a secondary consequence of adaptation to new ecological niches (29, 30). Our results demonstrate the reverse relationship, in which brain evolution directly promotes diversification. We reveal that evolutionary change in the functional organization of sensory pathways can establish new perceptual abilities that trigger explosive diversification.

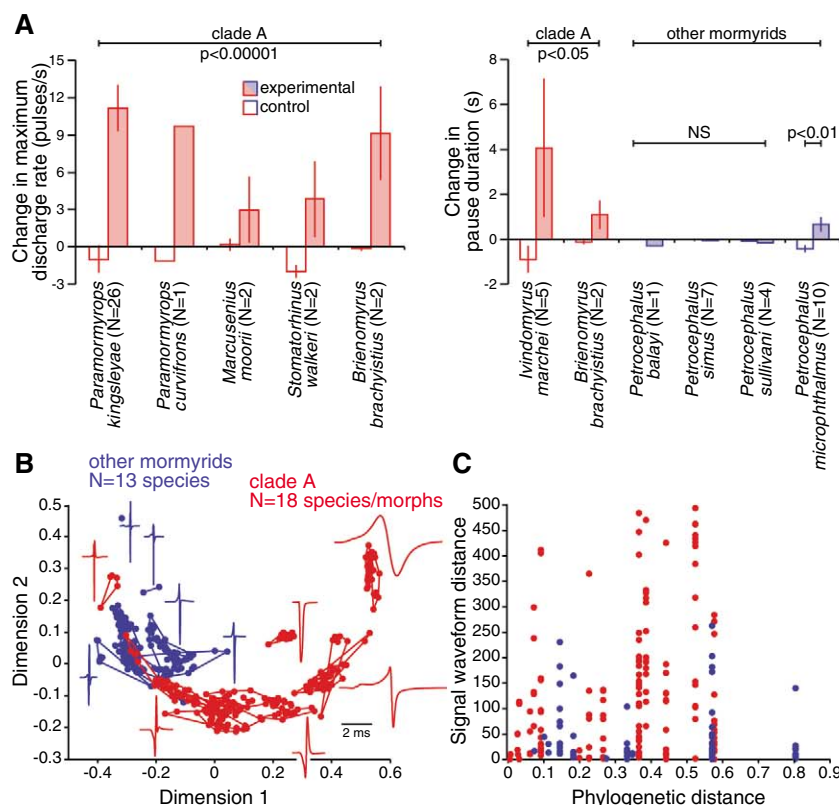


Fig. 4. Behavioral discrimination of signals and the evolution of signal diversity. **(A)** Dishabituation of behavioral responses, shown as the change in response from the eighth to ninth stimulus (mean $\pm$ SEM; see text). Species in clade A are shown in red; all other species are shown in blue. Statistical significance was assessed using Wilcoxon's matched-pairs test. **(B)** Minimum polygons enclosing species or morphs in bivariate signal space (as obtained through multidimensional scaling of signal cross-correlations; see SOM), including 11 example signals placed next to their location within this space. **(C)** Pairwise signal distances (Mahalanobis D^2) between species or morphs plotted against pairwise phylogenetic distances between *cytb* sequences.

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Supporting Online Material

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Self-Organizing and Stochastic Behaviors During the Regeneration of Hair Stem Cells

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Stem cells cycle through active and quiescent states. Large populations of stem cells in an organ may cycle randomly or in a coordinated manner. Although stem cell cycling within single hair follicles has been studied, less is known about regenerative behavior in a hair follicle population. By combining predictive mathematical modeling with in vivo studies in mice and rabbits, we show that a follicle progresses through cycling stages by continuous integration of inputs from intrinsic follicular and extrinsic environmental signals based on universal patterning principles. Signaling from the WNT/bone morphogenetic protein activator/inhibitor pair is coopted to mediate interactions among follicles in the population. This regenerative strategy is robust and versatile because relative activator/inhibitor strengths can be modulated easily, adapting the organism to different physiological and evolutionary needs.

Continuous stem cell (SC) regeneration is essential for the maintenance of many adult organs, for example, in the bone marrow, skin, and gastrointestinal tract. Although regenerative behavior within a single SC cluster such as the hair bulge (1) or intestinal villi (2) has been studied, it is largely unknown how the regenerative behavior in populations of these SC clusters is coordinated. During development, thousands of cells can self-organize into anatom-

ic structures and patterns by coordinating just a few morphogenetic signals (3), as seen in the periodic patterning of skin appendages (4, 5). We hypothesize that the regenerative cycling of adult organ SCs can be similarly coordinated by diffusible signals and self-organize into spatiotemporal regenerative patterns.

Hair offers a suitable experimental model because hair follicles (HFs) cycle through phases of growth (anagen) and rest (telogen) (6). SCs are clustered in hair bulges, making them easier to study than SCs in other organs, where they are usually scattered randomly (7) (fig. S1A). Growing hairs produce pigmentation patterns that allow simultaneous monitoring of the regenerative behavior of thousands of SCs (Fig. 1A) (8, 9). Additionally, the skin is flat, restricting interactions between HFs to two dimensions, further simplifying the analysis.

We developed a cellular automaton (CA) model consisting of a regular grid of automata, with one automaton representing one HF (fig. S1B) (10). The eight automata surrounding one automaton are defined as its neighbors. With time, the state of each automaton changes according to rules that

take into account the state of neighboring automata. Automata in certain states can interact, generating complex, self-organizing patterns based on a simple set of rules. Such patterning behavior can be globally modulated by simple rule changes in local automaton-to-automaton interactions (11).

To form regenerative patterns, activating signals among SCs should be able to spread and stop. This is possible when SCs can differentially respond to the same signal at different times of their regenerative cycle. We previously identified four functional phases in the hair regenerative cycle: signal-propagating (P) and nonpropagating phases (A), and phases refractory (R) and competent (C) to such signals (12). Telogen HFs in R phase cannot enter anagen because bone morphogenetic proteins (BMPs) in the surrounding skin macroenvironment keep hair SCs quiescent (12). Telogen HFs in C phase are devoid of these inhibitors and can enter anagen as long as the sum of intrinsic and extrinsic activators is above the threshold. Intrinsic activators are produced as the result of hair SCs and dermal papilla interactions. Extrinsic activators come from neighboring P-phase anagen HFs and represent a form of collective positive feedback. Thus, HFs can enter anagen in two ways: autonomously, depending on the level of intrinsic activation, or non-autonomously, when activators are delivered by the surrounding macroenvironment. The probability of anagen entry is based on the sum of these fluctuating inputs.

We used mathematical simulations to test the sufficiency and robustness of this model. We show that the CA model encompassing P→A→R→C cycling can reproduce the full spectrum of hair regenerative patterns observed in mice: formation of initiation centers, wave spreading, maintenance of borders, and border instability (Fig. 1B, fig. S2, and table S1).

For a model to be robust and capture conserved patterning principles, it should be capable of explaining the diverse regenerative patterns seen in mutant mice and other animals. The duration of each phase of the regenerative cycle depends on the relative strengths of activators and inhibitors (Fig. 1A). We suggest that dif-

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fusible signaling molecules used for regulating SC activities within the HF are coopted to mediate interactions between neighboring HFs. Activator-driven propagation of regenerative waves and inhibitor-driven halting of wave propagation can be potentiated by respective ligands or dampened by antagonists secreted between HFs by the skin macroenvironment. Together, HF and skin macroenvironment-derived ligands and antagonists should combine to produce unique signaling profiles that define properties of $P \rightarrow A \rightarrow R \rightarrow C$ phases (fig. S3). We assigned generic signaling profiles for each of the four phases in terms of the activator/inhibitor ratio: for P, high/low; A, high/high; R, low/high; and C, low/low (Fig. 1A). We undertook predictive modeling by using our CA framework to anticipate how regenerative patterns might be altered by changing the level of hypothetical activators or inhibitors (Fig. 1B, fig. S3, and table S1). The key prediction from this model is that the strength of SC coupling can be

weakened by either inhibitory pathway ligands or activating pathway antagonists.

Indeed, previously we showed that inhibitory BMP pathway ligands produced by the skin macroenvironment maintain telogen HFs in R phase and prevent them from being activated by the advancing regenerative wave (12). To identify activating pathway(s) involved in SC coupling, we began by matching expression patterns of various diffusible antagonists with the signature pattern predicted by the CA model: P, low; A, high; R, high; and C, low. *Dkk1* and *Sfrp4*, both diffusible WNT pathway antagonists, prominently fit this expression pattern (Fig. 2B and fig. S5). Simultaneously, multiple WNT ligands are expressed by anagen HFs (fig. S5B) (13) and, in principle, can serve as diffusible activators to mediate regenerative coupling between HFs. This scenario predicts that competing WNT ligands and antagonists produce distinct patterns of WNT signaling: WNT is activated in C-phase telogen

HFs adjacent to P-phase anagen HFs but not in the same C-phase HFs next to A-phase anagen HFs.

To gauge WNT signaling, we used *BAT-gal* and *cond-lacZ* WNT reporter mice, where lacZ expression marks canonical WNT pathway activation. Indeed, WNT reporter mice show these predictions to be true. Massive WNT pathway activation is seen in C-phase HFs ahead of the $P \rightarrow C$ regenerative wave front (fig. S8, I and K) but not along the A-C border (fig. S8L). According to CA model predictions, the activating pathway should demonstrate stochastic signaling in C- but not R-phase HFs, forming the basis for rare spontaneous $C \rightarrow P$ activation events. We found striking differences in WNT signaling between R and C phases. Although there is almost no WNT activation in R phase, both reporter mice show many stochastically distributed WNT-active telogen HFs in C phase (Fig. 2E and fig. S8). The majority of WNT-active HFs are solitary (4.9% in *BAT-gal* and 5% in *cond-lacZ*), but groups of two to four WNT-active HFs are very rare (0.23 to 0.01%) (table S3). This WNT activity is localized not to SCs but to adjacent dermal papillae (DPs) (fig. S8C). We found that 99.9% of all spontaneous WNT activation events in DPs do not translate into spontaneous anagen initiations. However, when WNT activation occurs in groups of five or more DPs simultaneously (<0.01%), it leads to new anagen activation (fig. S8J). This illustrates the stochastic nature of activation events predicted by the model and implies that HFs work synergistically toward successful anagen reentry.

We tested the functional role of WNT signaling locally with protein-coated beads and globally with transgenic mice. Local injection of Wnt3a beads induces new regenerative waves, whereas injection of *Dkk1* beads disrupts advancement of the existing regenerative wave (Fig. 2, C and D, and fig. S7). Furthermore, when Wnt7a is overexpressed in *K14-Wnt7a* mice, there is shortening of R phase (from 28 to 12 days) and enhanced SC activation, evidenced by many more spontaneous anagen initiation events and faster wave spreading compared with that seen in WT (Fig. 2A and figs. S6 and S10, B and C). In *cond-lacZ;K14-Wnt7a* mice, nearly 100% of DPs become WNT-active in C and even R phases (Fig. 2F and fig. S8, G and H), but no anagen reentry is observed in early R phase. A simultaneous decrease in inhibitory BMP signaling upon an $R \rightarrow C$ transition is essential for WNT-mediated anagen reentry.

By fulfilling multiple CA model predictions for a hypothetical activator, WNT signaling emerges as the key pathway for mediating regenerative coupling between hair SCs. Our data show that successful anagen reentry requires both down-regulation of BMP in the macroenvironment and spontaneous up-regulation of WNT activity in DPs (fig. S8M). (i) Loss of inhibitors (BMP ligands and WNT antagonists) from the skin macroenvironment in C phase enables HFs to express fluctuating spontaneous WNT activity in DPs. (ii) Such WNT activity in a single HF is

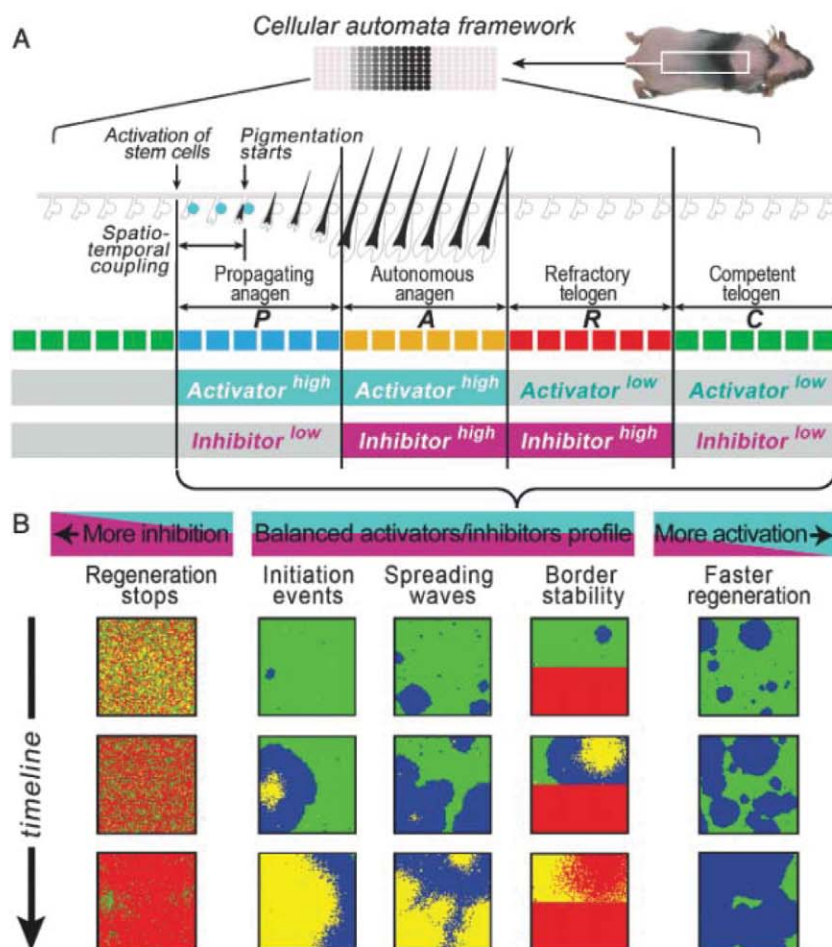


Fig. 1. A two-dimensional CA model can predict regenerative patterns in a large population of hair SCs. (A) Skin pigmentation patterns result from color changes of many HFs when they collectively cycle through four phases: P (blue)→A (yellow)→R (red)→C (green). Distinct hypothetical activator/inhibitor signaling profiles can be assigned to all four phases. (B) A two-dimensional CA model, in which each color-coded element (automaton) represents a single HF (fig. S1B), can reproduce regenerative patterns observed in mice: spontaneous initiation, spreading waves, and stability of borders. The model can also predict changes in patterns when there are either more inhibitors or more activators.

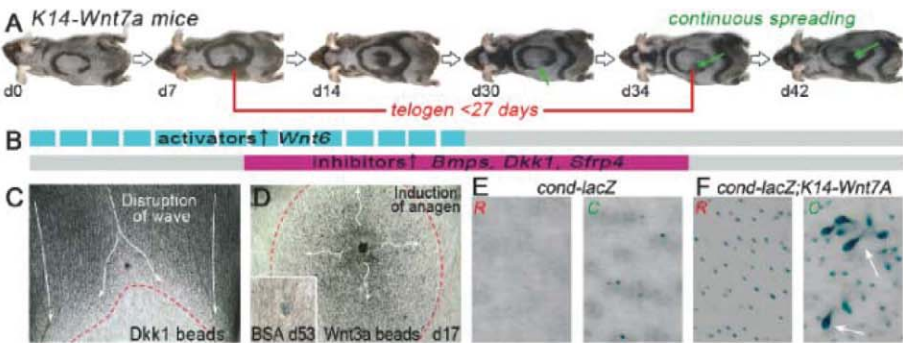


Fig. 2. WNT signaling plays an activating role in the coordinated regeneration of hair SCs in a follicle population. (A) Wnt7a overexpression in *K14-Wnt7a* mice results in regenerative patterns with shortened R phase, multiple spontaneous initiation centers, fast wave spreading, and lack of border stabilization (figs. S6 and S10). (B) Differential expression of key activators and inhibitors in the skin macroenvironment (data in fig. S5). (C and D) In bead implantation experiments, Wnt3a induces a new regenerative wave (D), whereas Dkk1 disrupts spreading of the existing regenerative wave (C) and control bovine serum albumin (BSA) has no effect (D, inset) (fig. S7). White arrows show directions of anagen spreading waves; red outlines mark anagen-telogen boundaries. (E and F) In *cond-lacZ* WNT reporter mice, spontaneous WNT activity (blue dots) occurs in DPs in C-phase but not R-phase telogen HF. Large clusters of WNT-active DPs are very rare (table S3). In *cond-lacZ;K14-Wnt7a* mice, 100% of DPs become WNT-active throughout telogen, which induces many new anagen initiation events (white arrows) during C phase (fig. S8).

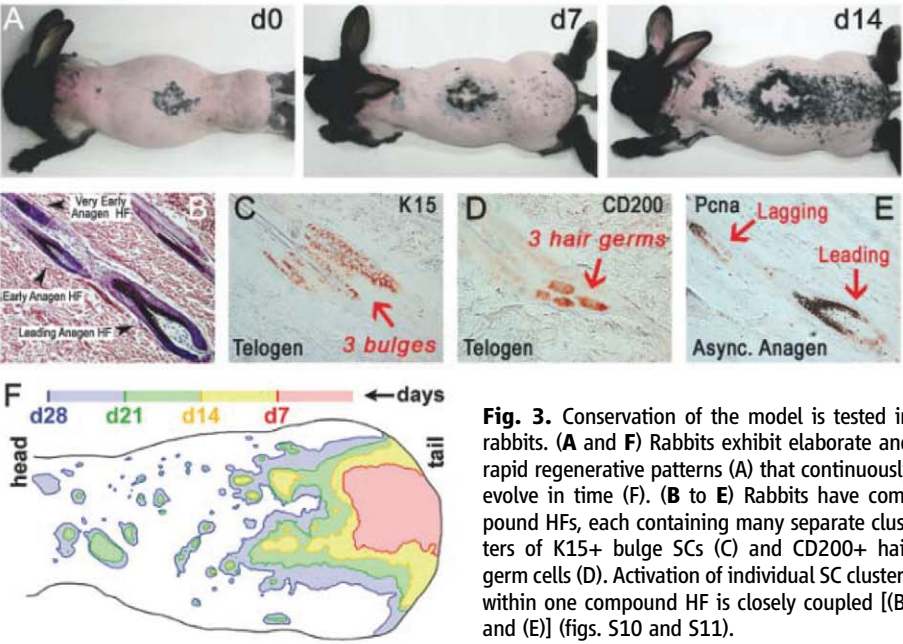
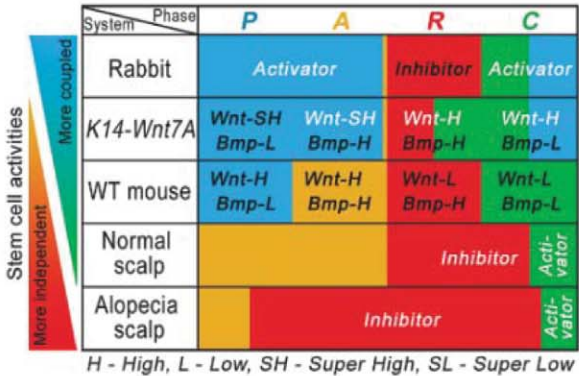


Fig. 3. Conservation of the model is tested in rabbits. (A and F) Rabbits exhibit elaborate and rapid regenerative patterns (A) that continuously evolve in time (F). (B to E) Rabbits have compound HF, each containing many separate clusters of K15+ bulge SCs (C) and CD200+ hair germ cells (D). Activation of individual SC clusters within one compound HF is closely coupled [(B) and (E)] (figs. S10 and S11).

Fig. 4. A unifying model of coordinated regeneration of hair SCs. The CA model predicts how simple changes in the relative levels of activators and inhibitors change SC coupling efficiency and modulate duration of P→A→R→C phases (colors are based on Fig. 1A). This produces versatile hair regenerative patterns that help animals adapt to different physiological conditions. See fig. S16.



not sufficient to drive anagen reentry. When several DPs adopt a WNT-active status simultaneously, they reinforce each other, and C→P activation occurs stochastically. Indeed, WNT signaling in DPs was recently shown to be critical for their anagen-inducing effect, likely mediated by secondary FGF signaling (14). Alternatively, in regenerative waves the wave front carries many activators that induce all C-phase HFs to enter anagen.

Compared with mice, rabbits have more robust hair growth. They have compound HFs (fig. S12) (15), each containing multiple tightly packed SC clusters and DPs (Fig. 3, B to E, and fig. S11). The skin surface area of rabbits is also 30 times larger than in mice. We wanted to examine regenerative patterns in rabbits and see how our CA model fares against experimental data. We observed that C→P activations in all SC clusters within one compound HF are closely coupled, and in the context of our CA model they behave as one “supercluster” (one automaton) of SCs. Rabbits display complex, fractal-like regenerative patterns (Fig. 3, A and F, and figs. S9 and S10) closely reminiscent of patterns generated by our model (fig. S4A). By exploring their fractal geometry, we show that large pieces of regenerative patterns in rabbits remain geometrically similar to much smaller pieces of themselves (fig. S14). Thus, the same CA principles are effective in managing regeneration of hair SCs regardless of their total number, arrangement, or organ size. Notably, pattern-forming signaling cues are likely conserved between mice and rabbits, because activation events readily spread from rabbit to mouse HFs in the cross-species grafting system, resulting in hybrid patterns (fig. S13).

Our modeling predicts that substantial increases in inhibiting signals or decreases in activating signals would reduce and eventually prevent coupling among HFs (Figs. 1B and 4). We see this in humans, where regenerative waves are observed in fetal scalp during the first two cycles (16) but disappear in the adult, when all HFs cycle independently and randomly (17). In adults, the lack of SC coupling makes hair regeneration depend solely on intrinsic activation mechanisms, making it particularly vulnerable to any decreases in intrinsic SC activation. Without coupling, such decreases can ultimately lead to baldness as seen in androgenic alopecia and “short anagen syndrome” (figs. S15 and S16A) (18).

We have shown how organ-wide SC management can be achieved. We speculate that during evolution integration of signals from single to multiple HFs across skin likely facilitated the formation of new mechanisms of regeneration. SC clusters can now be regulated as one entity, allowing organ regeneration to occur episodically with an intrinsic rate (fig. S16B, y axis). Cooption of key WNT and BMP signaling pathways from HFs by the skin macroenvironment allows for coupling between the SC clusters (fig. S16B, x axis). We conjecture that such a mechanism provides animals with a simple, yet robust and effective, way to coordinate the regeneration of very large SC pop-

ulations, which would otherwise be impossible with an intrinsic activation mechanism alone. We observe that regenerative hair patterns can differ in the same animal under different physiological conditions, allowing organisms to adapt to the environment (e.g., pregnancy in mice) (12). At the evolutionary scale, macroenvironmental regulation makes hair growth a trait that has high modularity. Lastly, beyond HFs, the experimental accessibility of this system offers a model for analyzing the fundamental principles of self-organizing behaviors in biological systems composed of coupled cycling elements.

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Supporting Online Material

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Conserved Eukaryotic Fusogens Can Fuse Viral Envelopes to Cells

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Caenorhabditis elegans proteins AFF-1 and EFF-1 [C. *elegans* fusion family (CeFF) proteins] are essential for developmental cell-to-cell fusion and can merge insect cells. To study the structure and function of AFF-1, we constructed vesicular stomatitis virus (VSV) displaying AFF-1 on the viral envelope, substituting the native fusogen VSV glycoprotein. Electron microscopy and tomography revealed that AFF-1 formed distinct supercomplexes resembling pentameric and hexameric “flowers” on pseudoviruses. Viruses carrying AFF-1 infected mammalian cells only when CeFFs were on the target cell surface. Furthermore, we identified fusion family (FF) proteins within and beyond nematodes, and divergent members from the human parasitic nematode *Trichinella spiralis* and the chordate *Branchiostoma floridae* could also fuse mammalian cells. Thus, FF proteins are part of an ancient family of cellular fusogens that can promote fusion when expressed on a viral particle.

Membrane fusion is critical for many biological processes such as fertilization, development, intracellular trafficking, and viral infection (1–6). Current models of the molecular mechanisms of membrane fusion rely on experimental and biophysical analyses performed on viral and intracellular, minimal, fusion-mediating machineries. Yet, how well these models correspond to the mechanisms of cell-cell

fusion is unknown (4, 5). *Caenorhabditis elegans* fusion family (CeFF) proteins were identified as C. *elegans* fusogens that are expressed at the time and place of cell fusion in vivo (7, 8). Expression of CeFF proteins is essential for developmental cell fusion via hemifusion and sufficient to fuse cells in vivo and in insect cell cultures (8–10).

To identify putative fusion family (FF) members in other species, we conducted sequence comparisons (4, 11). These comparisons yielded putative members in 35 nematodes, two arthropods (*Calanus finmarchicus* and *Lepeophtheirus salmonis*), a ctenophore (*Pleurobrachia pileus*), a chordate (*Branchiostoma floridae*), and a protist (*Naegleria gruberi*) (Fig. 1A). FF proteins are putative members of the “mostly β sheet super family” and share a pattern of cysteines, implying that they are conserved at the level of structure (fig. S1).

To determine whether divergent FF proteins maintained their function as fusogens through evolution, we expressed FF proteins from the human parasitic nematode *Trichinella spiralis*

(*Tsp-ff-1*) and the chordate *B. floridae* (*Bfl-ff-1*) in baby hamster kidney (BHK) cells and compared their fusogenic activity to AFF-1 (Fig. 1, B to F). These orthologs share 26 and 22% sequence identity with AFF-1, respectively. We observed, by immunofluorescence, $28 \pm 4\%$ and $37 \pm 7\%$ multinucleation in cells transfected with *Tsp-ff-1* and *Bfl-ff-1*, compared with $26 \pm 2\%$ and $4 \pm 3\%$ multinucleation in controls transfected with *aff-1* and empty vector, respectively (Fig. 1F) (11). In addition, when we expressed the EFF-1 paralog from the nematode *Pristionchus pacificus* in C. *elegans* embryos, we detected ectopic fusion of cells that normally do not fuse (fig. S2). Thus, FF proteins represent a conserved family of cellular fusogens.

To explore whether FF proteins can functionally substitute for viral fusogens, we complemented VSVΔG pseudoviruses with AFF-1 (Fig. 2 and fig. S3). We initially used a recombinant vesicular stomatitis virus (VSV) called VSVΔG, in which the glycoprotein G (VSVG) gene was replaced by a green fluorescent protein (GFP) reporter, to infect BHK cells overexpressing VSVG (11–16). The resulting VSVΔG-G viruses were capable of only a single round of infection, manifested by the production of GFP. We achieved complementation with AFF-1 by VSVΔG-G infection of BHK cells expressing AFF-1 (BHK-AFF-1), which generated pseudotyped particles carrying the nematode fusogen (VSVΔG-AFF-1). We biochemically validated incorporation of AFF-1 into VSVΔG pseudotypes by SDS-polyacrylamide gel electrophoresis, Coomassie staining, silver staining, immunoblotting, and mass spectrometry (11). We found that the major proteins on VSVΔG-AFF-1 were the viral proteins N, P, L, M, and AFF-1. For comparison, we also analyzed VSVΔG-G and VSVΔG (fig. S4 and table S5). Infection of BHK-AFF-1 cells with VSVΔG-AFF-1 showed a 600-fold increase compared with infection of BHK control cells not expressing AFF-1 (Fig. 2A). Although infection due to residual VSVG-complemented VSVΔG (VSVΔG-G) was negli-

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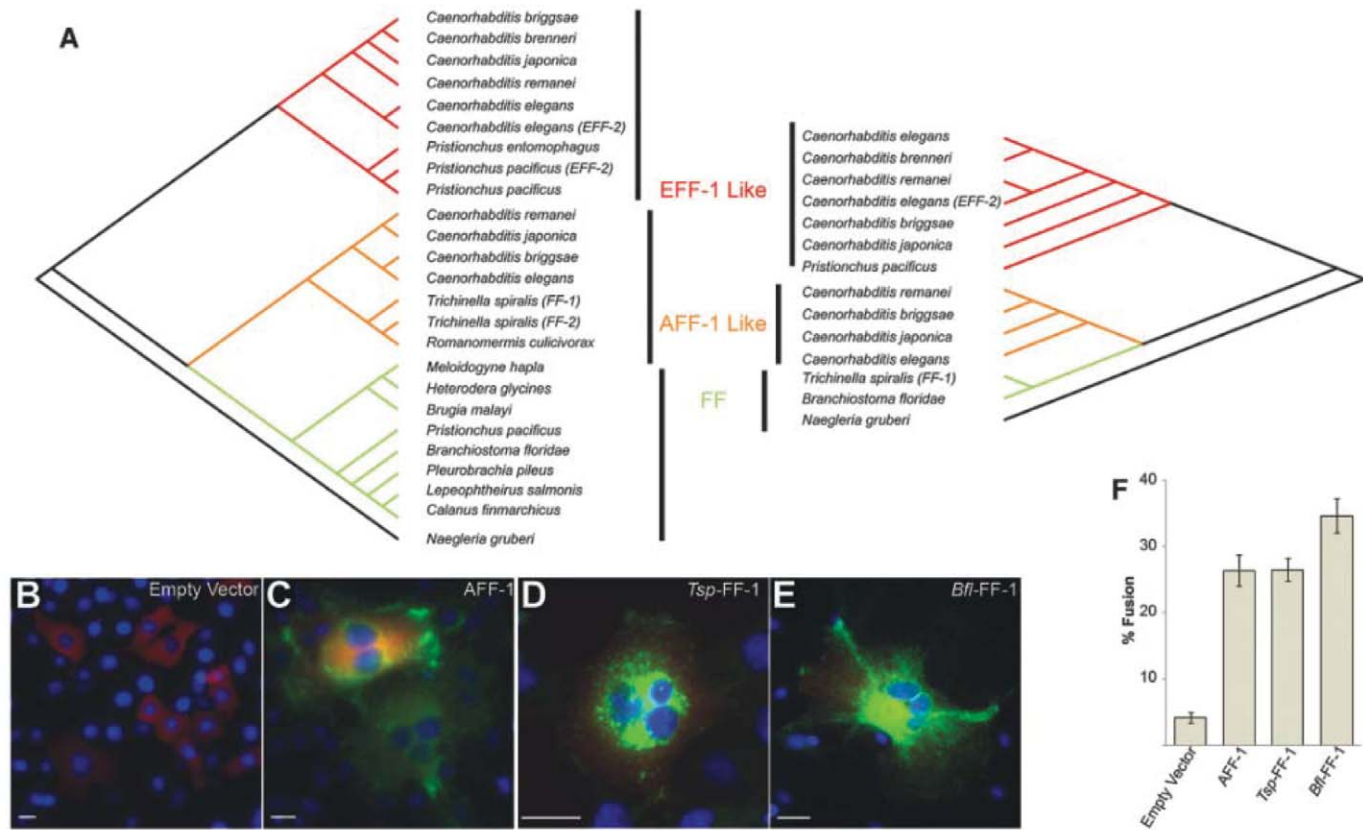


Fig. 1. A family of eukaryotic cell-cell fusogens: FF orthologs from two phyla fuse mammalian BHK cells. **(A)** Two trees produced using maximum parsimony analysis show phylogenetic relationships of 25 taxa [(left) based on the transforming growth factor- β -receptor type I-like domain (fig. S1B)] and 14 taxa [(right) based on the full-length extracellular domain]; FF proteins are classified into three subgroups: EFF-1-like (red), AFF-1-like (orange), and FF (green) (table S1). Consistency, retention, and composite indexes are detailed in (11). **(B)**

to **E)** Immunofluorescence with anti-Flag antibodies (green) and nuclei 4',6-diamidino-2-phenylindole staining (blue) on BHK cells transfected with **(B)** empty vector, **(C)** *aff-1*, **(D)** *Tsp-ff-1*, and **(E)** *Bfl-ff-1*. Cotransfection marker is shown in red. Scale bars, 20 μ m. **(F)** Fusion index for BHKs expressing FF proteins and negative control (empty vector). Data are means $\pm$ SE (error bars). Empty vector, $n = 14$; *aff-1*, $n = 14$; *Tsp-ff-1*, $n = 8$; *Bfl-ff-1*, $n = 9$ (n represents the number of experiments).

gible (Fig. 2), we performed inoculations in the presence of a neutralizing monoclonal antibody to G (anti-G antibody mAb I1) (17) to assure that we only measured AFF-1-mediated infection (fig. S5). Thus, AFF-1 can replace the viral fusogen VSVG and can mediate virus-to-cell binding and fusion.

VSV Δ G-AFF-1 could also infect cells expressing EFF-1 (BHK-EFF-1) (Fig. 2A) with comparable efficiency, suggesting that different CeFF proteins can functionally interact to mediate membrane fusion. To test this hypothesis, we evaluated cytoplasmic mixing between cells. We coexpressed *aff-1* with a red fluorescent protein containing a nuclear export signal (RFPnes) (Fig. 3) and mixed them with cells coexpressing *eff-1* and a cyan fluorescent protein containing a nuclear localization signal (CFPnl) (18). We cocultured the two cell populations and observed multinucleated cells expressing both markers (Fig. 3). In contrast, we did not observe cells expressing both markers among cocultured cells that were cotransfected with empty vector (Fig. 3A). Thus, AFF-1 and EFF-1 can promote heterotypic membrane fusion. To show independently that these results were a consequence of fusion, we recorded

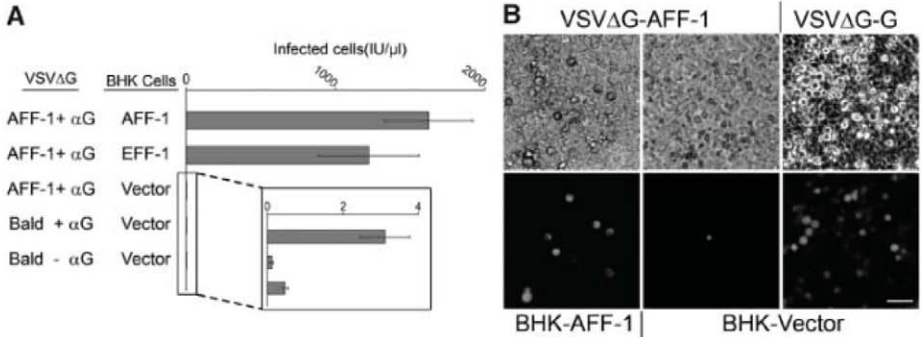


Fig. 2. AFF-1 can complement the infection of a fusion-deficient VSV Δ G. **(A)** Titers of VSV Δ G pseudoviruses. The type of protein on the viral membrane (bald or AFF-1) and on the BHK cell membrane (vector, AFF-1, or EFF-1) is indicated (fig. S3). Anti-VSVG antibody (α G) was used to neutralize any residual VSV Δ G-G virus (fig. S5) (11). Titers are measured in infectious units (IU), representing the number of cells expressing GFP per microliter 24 hours after virus inoculation. Data are mean $\pm$ SE (error bars; $n = 3$ experiments). (Inset) Background infection. We found no significant difference between infection of BHK-AFF-1 and BHK-EFF-1 (two-tailed paired t test, $P = 0.5841$). **(B)** Infection of BHKs monitored as GFP expression. (Top) phase contrast; (bottom) fluorescence. VSV Δ G-G served as positive control (fig. S5). Scale bar, 50 μ m.

time-lapse images of BHK-AFF-1 cells (fig. S6 and movies S1 and S2), supporting the conclusion that AFF-1 expression was enough to fuse cells.

Hence, AFF-1 and EFF-1 can mediate cell-cell fusion as well as viral-cell fusion by a CeFF-mediated mechanism. However, the VSV Δ G-

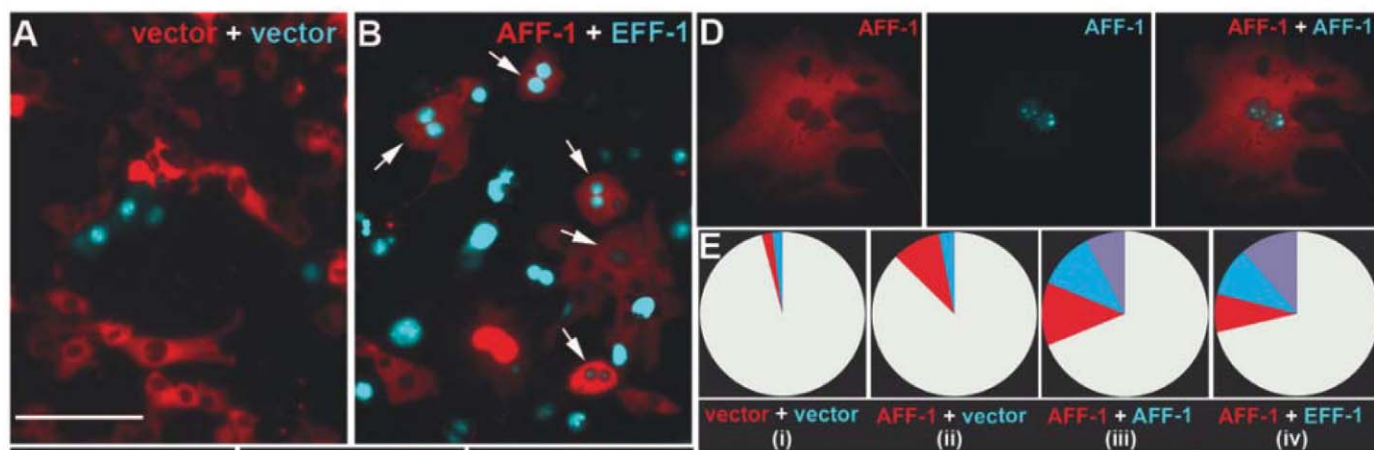


Fig. 3. BHK–AFF-1 can fuse with BHK–EFF-1 cells. **(A)** Negative control. Mixed cells cotransfected with empty vector and RFPnls or CFPnls. Scale bar, 100 μ m. **(B)** BHK–AFF-1 (red) and BHK–EFF-1 (cyan blue) cells were mixed. Hybrids express cyan blue nuclei and red cytoplasm (arrows). **(C)** BHK cell with red cytoplasm surrounding two cyan blue nuclei appeared after AFF-1–EFF-1 expression and mixing of the cells. Scale bar, 10 μ m. **(D)** Hybrid binucleate cell appeared after AFF-1 expression and mixing of the cells. **(E)**

Quantification of content mixing experiments. Red, cyan blue, and purple pie sections represent the fraction of multinucleated cells (two nuclei or higher). Results are mean of four independent experiments ($n \geq 1000$ total cells). (i) Empty vector transfected cells only. All multinucleated cells were binucleated (red or cyan blue, not purple); total binucleate cells = 4%, probably dividing cells. (ii) AFF-1–expressing cells (red) mixed with empty vector transfected cells (cyan blue). Elevation in multinucleation was only observed for AFF-1–expressing cells (red, 11%; cyan blue, 3%). One cell with a single nucleus expressing both markers (red and cyan blue) was observed. (iii) AFF-1–expressing cells (red) mixed with AFF-1–expressing cells (cyan blue), resulting in four cell populations: mononucleated (white, 64%), multinucleated (red, 13%; cyan blue, 12%), and mixed (purple, 11%). (iv) AFF-1–expressing cells (red, 9%) mixed with EFF-1–expressing cells (cyan blue, 11%). AFF-1– and EFF-1–expressing cells fuse (purple, 18%).

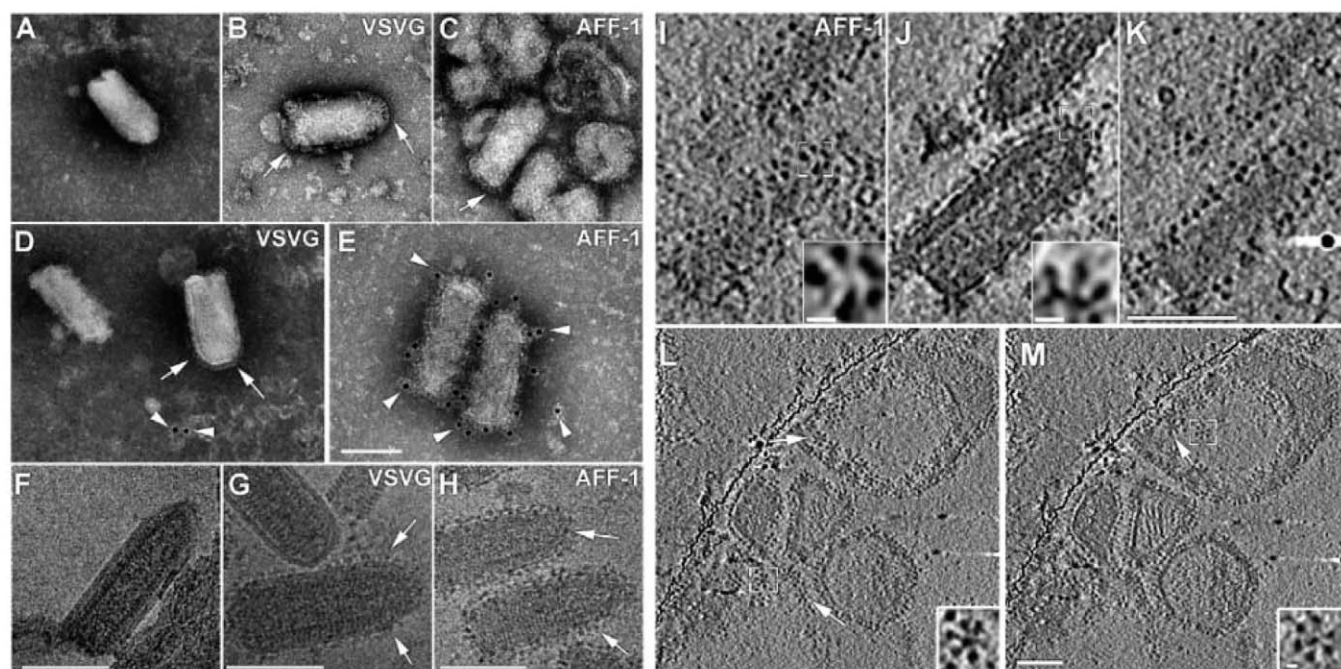


Fig. 4. EM of VSV Δ G–AFF-1 reveals specific bulky surface spikes. **(A to C)** Negative-stained particles of **(A)** VSV Δ G, **(B)** VSV Δ G–G, and **(C)** VSV Δ G–AFF-1. **(D and E)** Anti–AFF-1 polyclonal antibodies followed by immunogold labeling and negative stain of **(D)** VSV Δ G–G and **(E)** VSV Δ G–AFF-1 (figs. S7 and S8). **(F to H)** Cryo-EM of **(F)** VSV Δ G, **(G)** VSV Δ G–G, and **(H)** VSV Δ G–AFF-1. **(I to K)** Top, center, and bottom slice, respectively, from

VSV Δ G–AFF-1 tomogram (movie S3). **(L and M)** Slices from cryo-ET of vesicles copurified with VSV Δ G–AFF-1 preparations displaying penta- or hexameric flower-shaped assemblies (movie S5). Scale bars, 100 nm (main panels); 10 nm (insets). Arrows, surface spike assemblies; arrowheads, gold particles. The white squares in **(I)**, **(J)**, **(L)**, and **(M)** indicate the areas shown magnified in the insets.

AFF-1 infection mechanism is fundamentally different from that of native VSV. Whereas the infection of VSV is mediated by VSVG only on the viral membrane, infection mediated by VSVΔG–AFF-1 requires an FF protein on both the viral membrane and the cell membrane.

To study the relation between structure and function of AFF-1, we used transmission electron microscopy (TEM). We compared negatively stained samples of VSVΔG to VSVG- and AFF-1-complemented VSVΔG preparations (11). VSVΔG virions have the typical VSV shape with a smooth membrane (hence, they are termed “bald”), whereas both VSVΔG-G (19) and VSVΔG–AFF-1 virions displayed distinct spikes on their envelopes (Fig. 4, A to C). In negative stain (pH 5), VSVG forms elongated spikes on VSVΔG-G (Fig. 4B) (19), whereas VSVΔG–AFF-1 showed shorter spikes (Fig. 4C). The estimated average spike lengths of VSVG and AFF-1 were 145 and 109 Å, respectively (table S2). To confirm that the observed spikes were indeed AFF-1, we used anti-AFF-1 polyclonal antibodies to perform immunogold labeling. We observed specific immunoreactivity on the surface of VSVΔG–AFF-1 (Fig. 4, D and E, and figs. S7 and S8). To further characterize the pseudoviruses at higher resolution and in a more native state, we used cryogenic TEM (cryo-TEM) (Fig. 4, F to H) and cryogenic electron tomography (cryo-ET) (Fig. 4, I to K, and movie S3) to image them embedded in vitreous ice. Cryo-TEM projection images showed that AFF-1 proteins coat the pseudoviruses. Side views of individual spikes could be observed at central sections of the tomograms

(Fig. 4J). Higher-order assemblies of AFF-1 in the form of penta- or hexameric “flower-shaped” supercomplexes could be observed in slices through the tomogram oriented peripheral to the pseudotyped virus particles (Fig. 4I). These assemblies were more visible in slices through the tomograms of copurified vesicles (Fig. 4, L and M, fig. S9, and movies S4 and S5). The order of these arrays may have a critical function in bending and deforming plasma membranes to mediate fusion.

Here, we have presented evidence that FF proteins are functionally conserved in evolution and can restore the infectivity of VSVΔG through interactions with FF proteins on the target cell. Thus, FF, viral, and intracellular fusogens converge functionally as minimal fusion machines that function on their own to promote fusion.

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Supporting Online Material

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Movies S1 to S5

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The Spatial Periodicity of Grid Cells Is Not Sustained During Reduced Theta Oscillations

Julie Koenig, Ashley N. Linder, Jill K. Leutgeb, Stefan Leutgeb*

Grid cells in parahippocampal cortices fire at vertices of a periodic triangular grid that spans the entire recording environment. Such precise neural computations in space have been proposed to emerge from equally precise temporal oscillations within cells or within the local neural circuitry. We found that grid-like firing patterns in the entorhinal cortex vanished when theta oscillations were reduced after intraseptal lidocaine infusions in rats. Other spatially modulated cells in the same cortical region and place cells in the hippocampus retained their spatial firing patterns to a larger extent during these periods without well-organized oscillatory neuronal activity. Precisely timed neural activity within single cells or local networks is thus required for periodic spatial firing but not for single place fields.

Brain oscillations are thought to be essential for neural computations and for organizing cognitive processes (1–5). Yet it has been difficult to distinguish computations in neural circuits that require oscillatory neural activity from those that occur irrespective of the precise temporal organization that oscillatory rhythms can

provide. Theta oscillations (4 to 11 Hz) can be recorded in the local field potential of the hippocampus and the parahippocampal cortices, including the entorhinal cortex. These brain regions are important for memory and navigation (6) and contain a number of different cell types with precise spatial firing patterns, such as place cells,

head-direction cells, and grid cells (7–9). Grid cells are found in the medial entorhinal cortex (MEC), parasubiculum, and presubiculum and have multiple firing peaks that form a highly regular hexagonal firing pattern in two-dimensional space (8, 10).

The coexistence of cells with well-defined spatial firing patterns and of theta oscillations that are particularly prominent during voluntary movement (11) suggests that oscillatory brain activity might be essential for spatial computations. In particular, it has been proposed that precisely tuned theta oscillations, as an animal moves through its environment, might be necessary for generating the periodic spatial firing of grid cells. Such spatial regularity might arise from interference between oscillators with small frequency differences, given that those oscillators are controlled by both movement velocity and movement direction (12–14). Although the oscillators could be implemented in various ways in single neurons or

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Fig. 1. Field theta oscillations in the MEC and hippocampus were reduced after microinfusion of lidocaine into the medial septal area [red arrows in (A) to (C) and (E)]. **(A)** Behavioral design. Each of the four 10-min recording sessions is referred to as 1 to 4. **(B)** Time-frequency plots and raw LFP traces show the simultaneous reduction in theta power in the MEC and hippocampus. The raw traces are from periods with running speeds above 15 cm/s. **(C)** For experiments with recordings of cells in either the MEC (black) or hippocampus (blue), the percent change in the power of LFP oscillations between 7 and 9 Hz is shown compared to baseline. Each 10-min recording was subdivided into two 5-min intervals (a and b). **(D)** The peak frequency of remaining oscillations in the theta range decreased (hippocampus: $t = 3.37$, $P < 0.01$; MEC: $t = 3.67$, $P < 0.01$). **(E)** Average running speed during all sessions with single-unit recordings. Because the running speed before and after the lidocaine infusion was the same ($t = -1.97$, n.s.), the reduced theta power was not a consequence of reduced movement velocity.

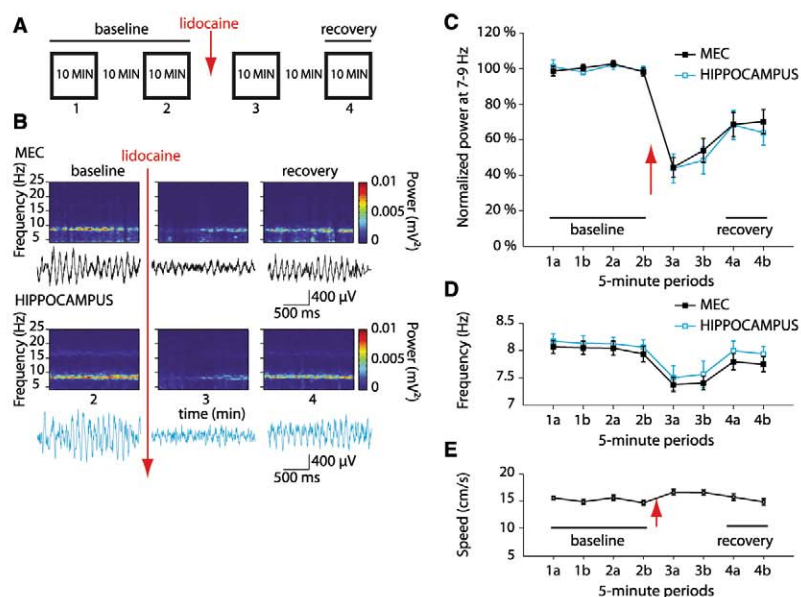
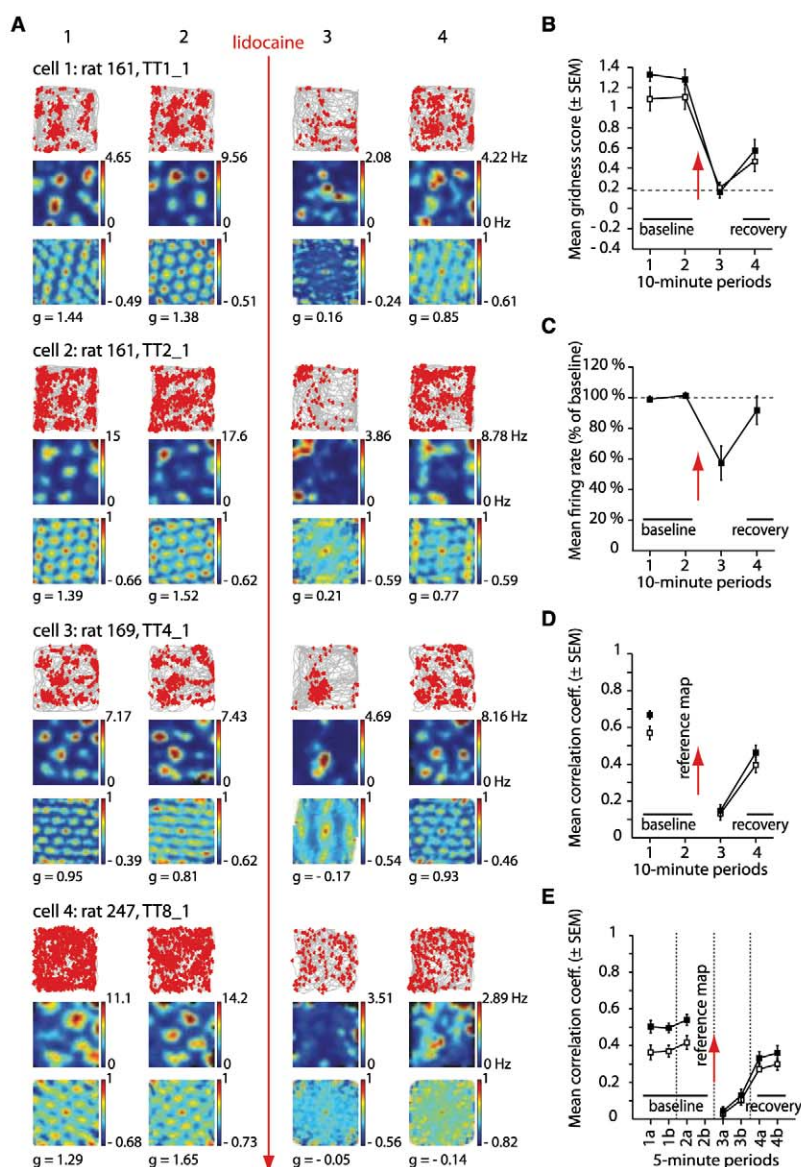


Fig. 2. The spatial periodicity of grid cells in the MEC vanished during periods of reduced theta activity. **(A)** Firing correlates for four representative MEC grid cells (see fig. S3 for additional examples). Cells 1 and 2 were recorded simultaneously. Trajectories (gray) with superimposed spike locations (red dots) are shown in the top row of each cell's panel. The corresponding color-coded rate maps and spatial autocorrelation matrices compose the middle and bottom rows, respectively. The color scale for rate maps (shown for each 10-min recording interval) is from 0 Hz to the peak rate, and for the spatial autocorrelation matrices is from the minimum correlation coefficient to 1. **(B)** In the original (solid symbols) and subsampled data (open symbols), the mean gridness score decreased to chance levels (stippled line) after septal inactivation ($n = 26$; $t = -0.64$ and 0.18 , n.s. compared to chance; $t = 11.89$ and 7.16 , $P < 0.001$ as compared to the score before inactivation). Although grid cell firing rates completely recovered after the infusion, the recovery of gridness was variable across the recorded population (see also fig. S6). **(C)** The mean firing rates for all recorded grid cells are shown as the percent change compared to baseline rates. Firing rates decreased after septal lidocaine infusions ($t = 3.19$, $P < 0.001$). **(D)** Spatial correlation coefficients between rate maps. The rate map for the session before lidocaine infusion (session 2) served as a reference map to which all others were compared, and therefore is not plotted. Grid cell firing after lidocaine infusion compared to baseline did not remain at corresponding locations after theta reduction ($t = 12.84$, $P < 0.001$). **(E)** During the 5-min period of the maximal drug effect, the spatial maps were completely uncorrelated to the period before the lidocaine infusion (original and subsampled data, $t = 2.04$ and 1.28 , n.s. compared to zero; $t = 11.8$ and $t = 9.92$, $P < 0.001$ compared to baseline).



local circuits, at least one of them would likely be phase-locked to theta oscillations. We tested the hypothesis that reducing theta oscillations by inactivating inputs from the subcortical pace-makers in the medial septal area (15–18) would disrupt the spatial computations of grid cells.

To explore the effects of reduced theta oscillations on spatial firing patterns, we trained 14 rats to randomly forage in a 1.2-m-by-1.2-m box and recorded the local field potential (LFP) and cells in the superficial layers of the MEC ($n = 90$ cells) and in the hippocampus ($n = 64$) during four consecutive 10-min recording sessions (Fig. 1A) (19, 20). After the first two 10-min sessions, we manipulated theta oscillations by microinfusions of lidocaine into the medial septum (fig. S1). Such infusions substantially reduced the amplitude and frequency of theta oscillations in the MEC and the hippocampus (Fig. 1 and fig. S2).

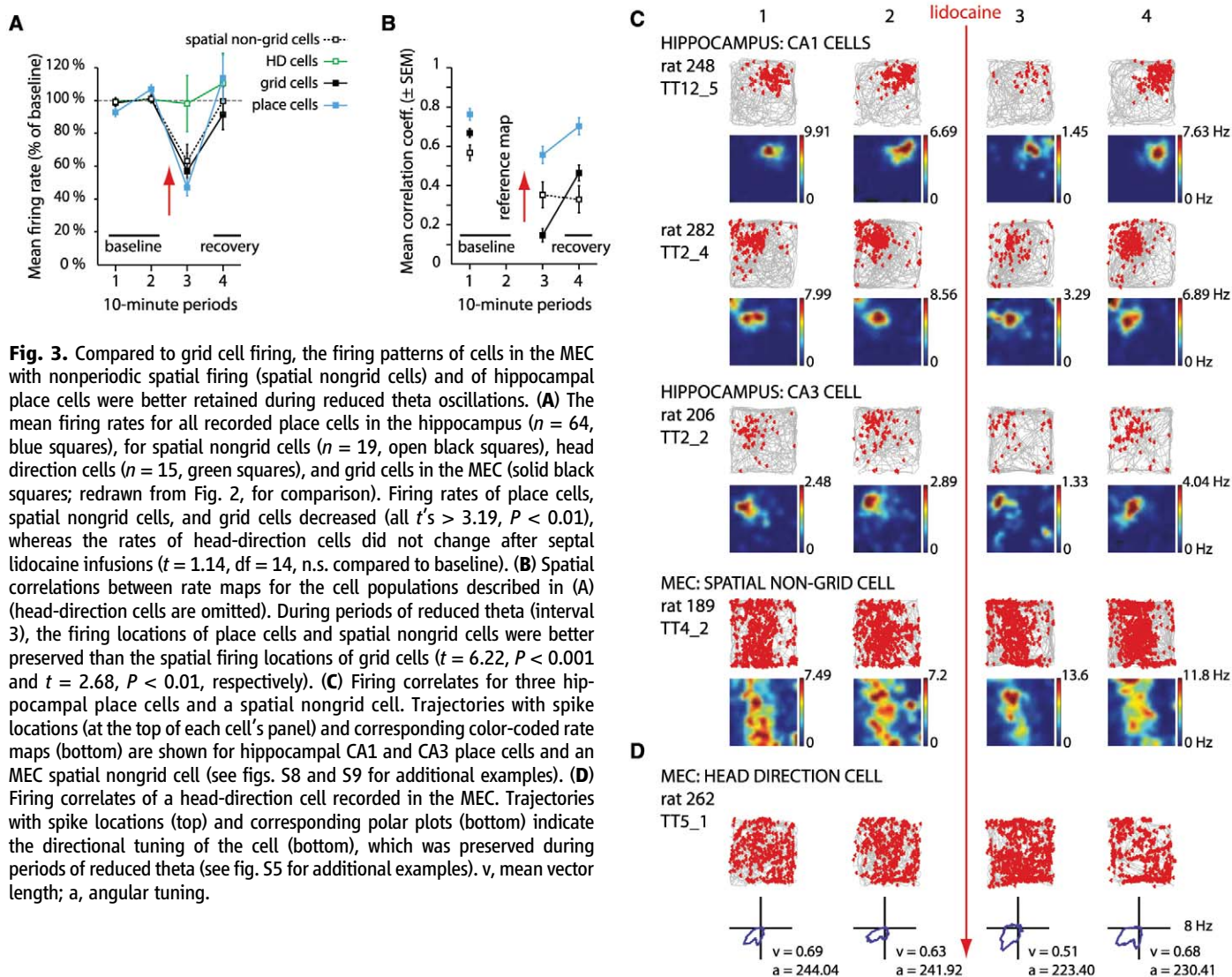
Would the spatial firing characteristics of cells predict their response to the manipulation? We first selected grid cells by measuring the hexagonal regularity of firing (i.e., “gridness”), only

accepting cells with scores higher than the 99th percentile of shuffled data. The periodic spatial firing pattern of grid cells (their gridness) vanished during the period of reduced theta (Fig. 2 and figs. S3 and S4). The firing rates of grid cells decreased during periods of theta reduction (Fig. 2C), whereas head-direction cells did not show changes in mean firing rate (Fig. 3, A and D) or mean vector length (fig. S5).

The parallel decrease in gridness and firing rates raised the question whether grid patterns in fact persisted but were obscured from detection because of the limited number of spikes. We therefore performed additional analysis. First, we observed that the firing rates of a subset of grid cells recovered in advance of their grid patterns and demonstrated that the two measurements were unrelated [correlation coefficient (r) = 0.07, n.s.; fig. S6]. Second, the spikes that remained during periods of reduced theta occurred not only without spatial regularity but also at locations unrelated to the original grid pattern (Fig. 2, D and E). Finally, we randomly omitted spikes from

recording periods with higher firing rates to obtain spike trains with matching average rates for each 10-min recording. As intended, this resulted in a low firing rate for each recording segment (0.82 ± 0.15 to 0.84 ± 0.15 Hz). We used these samples to recalculate gridness. As in the original sample, robust grid firing was seen in subsampled data before, but not after, theta oscillations were reduced (Fig. 2B and fig. S7).

Are theta oscillations thus required for sustaining any well-defined spatial firing? We selected MEC cells with spatial firing fields but without grid-like regularity [i.e., spatial nongrid cells (21–24)]. Their spatial firing locations were partially preserved, while their firing rates decreased to a similar extent as those of grid cells (Fig. 3, A to C, and fig. S8). In addition, many cells in the MEC continued to fire rhythmically as shown in autocorrelograms but at oscillation frequencies that decreased by 0.67 Hz ($t = 3.31$, $P < 0.01$). A similar decrease was seen for interneurons in the MEC and for hippocampal cells (fig. S2), indicating a downshift in the intrinsic oscillation frequen-



cy of the hippocampo-entorhinal circuitry (25) when pacing by septal inputs was reduced.

Hippocampal place cells are also spatially modulated cells without periodicity in their firing pattern. Their spatial firing patterns are partially affected in the radial maze after septal inactivation and in the open field during reduced cholinergic neurotransmission (26, 27). For comparisons with MEC cells, we therefore recorded from hippocampal place cells in the same experimental design while reducing theta oscillations to the same extent as in MEC recordings (fig. S2) (20). We observed a substantial decrease in the firing rate of hippocampal place cells, and the spike trains of individual hippocampal cells showed reduced theta modulation (Fig. 3A and fig. S2). Place fields remained well-defined and at corresponding locations (Fig. 3, B and C, figs. S9 and S10, and table S1).

By silencing the septal area, we diminished theta oscillations in the entorhino-hippocampal circuitry and showed that the periodic firing of grid cells does not persist. Subcortical inputs to hippocampus and parahippocampal cortices are thus essential not only for theta oscillations but also for sustaining the spatial periodicity of grid cells. These findings are consistent with the theory that grid cells emerge from the interference between multiple precisely tuned theta oscillations within individual cells (12–14). Alternatively, the silencing of septal inputs to the MEC might result in the desynchronization of grid cells, so that the local network of cortical cells can no longer generate oscillatory interference (12, 28). Our data also identified a subpopulation of grid cells that do not regain their spatial regularity when theta oscillations recover (fig. S6). This suggests that a fraction of grid cells might not be directly participating in the generation of grid patterns, but rather becomes associated with other grid cells by plasticity-dependent mechanisms.

A parallel study (29) has independently discovered that grid cell firing does not persist during reduced theta oscillations and that other cell types are less affected. Together, our results show that the neuronal network mechanisms that sustain the periodic spatial firing of grid cells are different from those required for other firing correlates in the entorhino-hippocampal circuitry, including head-direction cells and place cells. The effect on grid cells is likely not mediated through effects of septal silencing on the firing of hippocampal place cells, because it has been shown that grid cell firing initially remains intact after the hippocampus has been silenced (30). Subcortical inputs are therefore necessary for the neural computations in the MEC that generate grid-like, periodic, spatial firing patterns, whereas the firing locations of place cells largely persist after inputs from grid cells and from subcortical areas to the hippocampus have substantially changed.

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21. The firing fields of a subset of MEC cells had the stripe-like appearance of boundary vector cells, but only 6 of 19 fired maximally at the border. A field size

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Reduction of Theta Rhythm Dissociates Grid Cell Spatial Periodicity from Directional Tuning

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Grid cells recorded in the medial entorhinal cortex of freely moving rats exhibit firing at regular spatial locations and temporal modulation with theta rhythm oscillations (4 to 11 hertz). We analyzed grid cell spatial coding during reduction of network theta rhythm oscillations caused by medial septum (MS) inactivation with muscimol. During MS inactivation, grid cells lost their spatial periodicity, whereas head-direction cells maintained their selectivity. Conjunctive grid-by-head-direction cells lost grid cell spatial periodicity but retained head-direction specificity. All cells showed reduced rhythmicity in autocorrelations and cross-correlations. This supports the hypothesis that spatial coding by grid cells requires theta oscillations, and dissociates the mechanisms underlying the generation of entorhinal grid cell periodicity and head-direction selectivity.

The role of oscillations in neural coding is controversial. Theta frequency oscillations (4 to 11 Hz) play an important role in memory behavior (1–4) and code spatial location by the precession of spike timing relative to theta

oscillations (theta phase precession) in the hippocampus (5, 6) and medial entorhinal cortex (MEC) (7). However, disagreement remains about whether theta oscillations are critical to spatial coding by neurons. Grid cells (8, 9) in the MEC provide a powerful example for testing the theoretical role of oscillations in neural coding. Some computational models of grid cells use network theta rhythm oscillations to generate grid cell spatial periodicity (10, 11). These models simulate the phase of spike timing in grid cells (7)

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and have successfully predicted that the spatial scale of grid cell firing correlates with measures of intrinsic rhythmicity (12, 13). Recent models also show the potential role of theta oscillations for updating position in attractor dynamic models of grid cells (14). In rats, we tested the role of theta rhythm oscillations in the spatial coding of grid cells by testing the spatial periodicity of grid cells during pharmacological disruption of theta rhythm oscillations.

Lesions or inactivation of the medial septum (MS) in rats cause a disruption of theta oscillations in the entorhinal-hippocampal system (2, 4, 15, 16) and cause spatial memory impairments (1–4, 17, 18). We performed microinfusions of muscimol to pharmacologically inactivate the MS bilaterally (1, 17). Simultaneously, we used tetrodes in the MEC to monitor the spiking activity of grid cells, head-direction cells, and conjunctive grid-by-head-direction cells.

After infusions of muscimol into the MS (Fig. 1A, left), recordings in the MEC (Fig. 1A,

right) demonstrated a clear decrease in the power of theta oscillations in the MEC local field potential (Fig. 1B) and a strong reduction in the spatial periodicity of grid cells (Figs. 1, C and D, and 2A and fig. S3). Recovery of theta rhythm and spatial periodicity occurred in recordings 3 to 6 hours and 24 hours after the infusion. The “gridness” (hexagonal regularity of firing) score measured before the MS inactivation showed a significant decrease after the inactivation ($n = 29$ cells; baseline: mean $\pm$ standard error: 0.64 ± 0.05 gridness score; inactivation: -0.27 ± 0.06 gridness score, $P < 0.001$) (Fig. 3A) that recovered at 3 to 6 hours and 24 hours (3 to 6 hours: $n = 26$, 0.35 ± 0.10 gridness score; 24 hours: $n = 21$, 0.46 ± 0.09 gridness score). Because the firing rates of grid cells were also reduced ($n = 29$; baseline: 1.88 ± 0.20 Hz; inactivation: 1.17 ± 0.21 Hz, $P < 0.001$), we subsampled the spiking of the baseline recordings before infusion to match the same overall firing rate after infusion to con-

firm that the reduction of gridness still appeared when compared to subsampled data ($n = 29$; subsampled: 0.58 ± 0.05 gridness score; inactivation: -0.27 ± 0.06 gridness score, $P < 0.001$) (column 2 in Fig. 1, C and D, and fig. S3). There were no differences in running speeds between the baseline, MS inactivation, 3- to 6-hour, or 24-hour recovery periods (Fig. 4C and fig. S8). Control infusions of phosphate-buffered saline (PBS) into the MS did not alter theta oscillations or the grid cell spiking patterns in the MEC (Fig. 1E and fig. S7).

Many neurons in the MEC are conjunctive grid-by-head-direction cells (19, 20), head-direction cells (19, 21), or cells showing spatial selectivity without grid cell periodicity, such as border cells (22, 23). We observed a dissociation in the effect of MS inactivation on the spatial periodicity and head-direction specificity of entorhinal neurons. After muscimol infusion, conjunctive grid-by-head-direction cells fired with reduced spatial periodicity (Fig. 2A

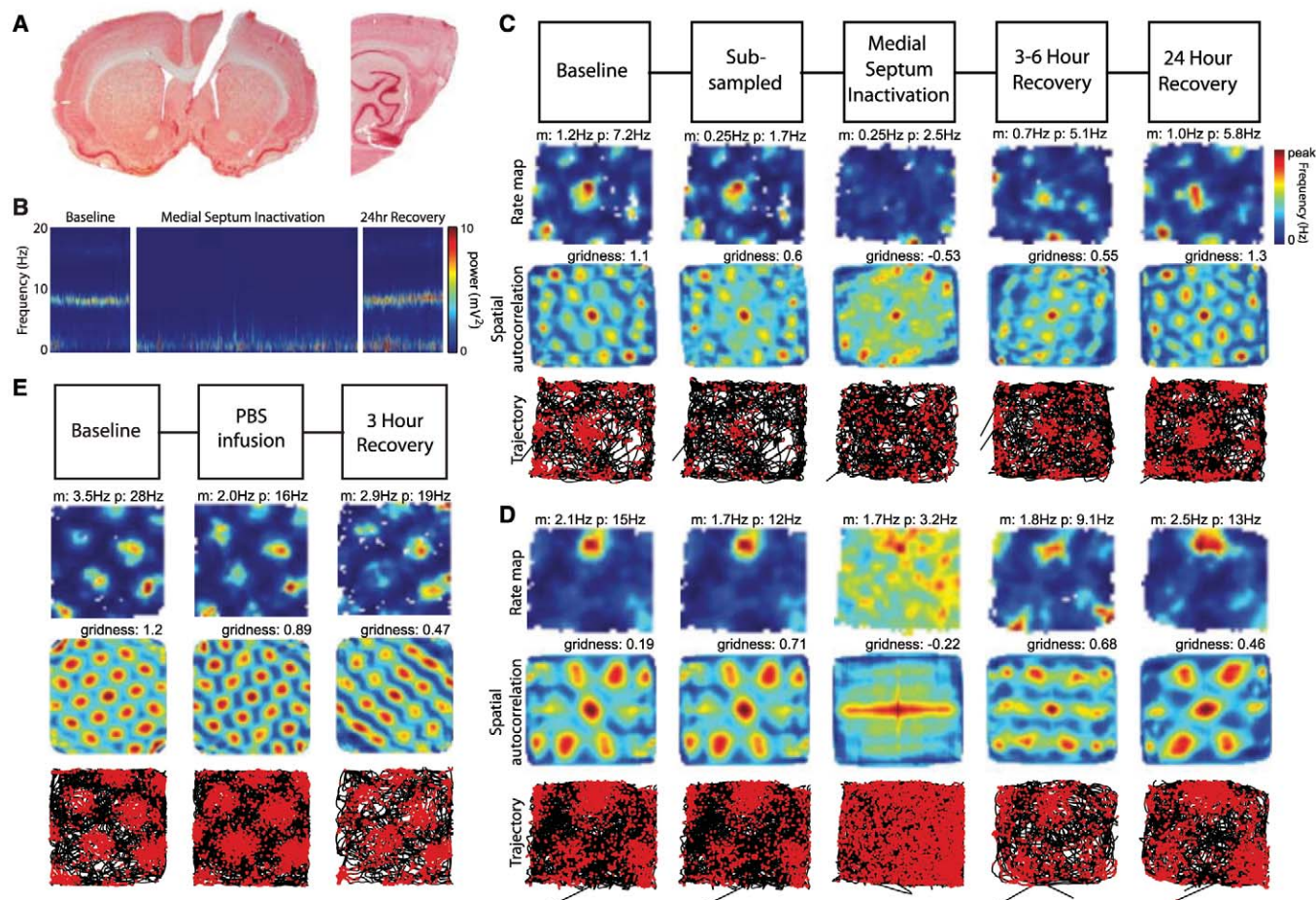


Fig. 1. Loss of grid cell spatial periodicity during reduced theta rhythm oscillations. (A) (Left) Muscimol infusions were delivered via a cannula in the MS. (Right) Tetrode tracks show the location of recording in the MEC. (B) Spectrogram of MEC local field potential showing loss of pre-infusion theta peak after infusion. (C) Column 1: Grid cell firing before infusion (Baseline). m, mean rate; p, peak rate. Column 2: Subsampling of pre-infusion firing rate to match rate 15 to 75 min after infusion. Column 3: Grid cell firing 15 to

75 min after muscimol infusion (Medial Septum Inactivation) demonstrating loss of grid cell spatial periodicity during reduced theta. Columns 4 and 5: Recovery 3 to 6 hours (3–6 Hour Recovery) and 24 hours (24 Hour Recovery) after infusion. Row 1, firing rate maps; row 2, autocorrelation maps; row 3, animal trajectory with spikes (red dots). (D) Example of another grid cell before and after infusion. (E) Example of grid cell firing before and after control infusion of PBS.

and fig. S3) but retained their directional preference, ϕ [$n = 8$, 1.72 ± 1.15 $|\Delta\phi|$ degrees, not significant (n.s.)] (Figs. 2A and 3B and fig. S3). After infusion, head-direction cells retained their directionality ($n = 11$; baseline: 37.89 ± 8.9 Watson U^2 statistic; inactivation: 39.55 ± 14.54 Watson U^2 statistic, n.s.; 24.64 ± 9.17 $|\Delta\phi|$ degrees, n.s.) (Figs. 2B and 3B and fig. S4) and as a population retained their firing rates ($n = 11$; baseline: 2.7 ± 0.72 Hz; inactivation: 1.9 ± 0.65 Hz, n.s.) (Fig. 3A). Cells with positive gridness scores showed a reduction of spatial information (grid cells: $n = 29$; baseline: 1.08 ± 0.07 bits per spike; inactivation: 0.56 ± 0.05 bits per spike, $P < 0.001$) (Fig. 3A). Cells with negative gridness scores but high spatial information also showed a reduction of spatial information (spatially modulated non-grid cells: $n = 9$; baseline: 1.3 ± 0.15 bits per spike; inactivation: 0.70 ± 0.10 bits per spike, $P < 0.01$) (Fig. 3A and fig. S5).

The slow onset and long half-life that are characteristic of muscimol effects (1, 17) permitted us to quantify the relationship between theta power and grid cell spiking. Using 10-min sliding window calculations, we found a clear similarity in the time course between theta power and gridness (Fig. 3D) and a consistent rela-

tionship between the power of theta and the gridness score (Fig. 3C). Could a drift in spatial phase or orientation explain the loss of the grid pattern after infusion? Across all of our recording sessions, large gridness scores did not appear during periods of low theta index (Fig. 3, C and D). A drifting grid cell representation would usually maintain the relative spike timing between simultaneously recorded grid cells. When multiple grid cells were recorded, they often showed rhythmic peaks in their spike time cross-correlations that were reduced or eliminated during MS inactivation (Fig. 4B), as shown by the reduction of power in the theta rhythm in cross-correlations, suggesting a reduction of temporal consistency. During MS inactivation, the cross-correlation plots reveal a reduction in percentage of theta rhythmic cells (baseline: 53%, inactivation: 23%, $n = 350$) and theta power ratio (baseline: 3.4 ± 0.01 , inactivation: 0.23 ± 0.01 , $n = 187$, $P < 0.001$). Auto-correlation plots show a reduced percentage of theta rhythmic cells (baseline: 38%, inactivation: 15%, $n = 78$) and autocorrelation theta power ratio (baseline: 4.1 ± 0.35 , inactivation: 1.37 ± 0.21 , $n = 30$, $P < 0.001$). A decrease in the frequency of remaining theta rhythmic cells appeared in both cross-correlations ($n = 62$; base-

line: 6.83 ± 0.02 , inactivation: 6.34 ± 0.06 , $P < 0.001$) and autocorrelations ($n = 10$; baseline: 7.10 ± 0.08 , inactivation: 6.66 ± 0.27) (Fig. 4, B and D). The frequency increase at 3 to 6 hours (Fig. 4D) may arise from rebound of currents after inactivation. Some autocorrelation plots also exhibited theta cycle skipping (24) during control conditions (Fig. 4B), yet lost this attribute during MS inactivation (baseline: $n = 30$, 60% of theta rhythmic cells; inactivation: $n = 13$, 0% of theta rhythmic cells; theta skipping index, $n = 12$, baseline: 0.08 ± 0.03 , inactivation: -0.12 ± 0.01 , $P < 0.001$).

These results are complementary to the results obtained independently by Koenig *et al.* (25). The use of muscimol here demonstrates the correlated reduction of theta power and gridness score over a long time course, and the use of lidocaine in the companion paper shows the relationship over a faster time course. The recording in deeper layers of the entorhinal cortex here demonstrates the sparing of head-direction selectivity in conjunctive cells and in pure head-direction cells, and the companion paper shows the sparing of hippocampal place cell specificity, consistent with previous studies (26). Our data show a reduction of rhythmicity in temporal cross-correlations between

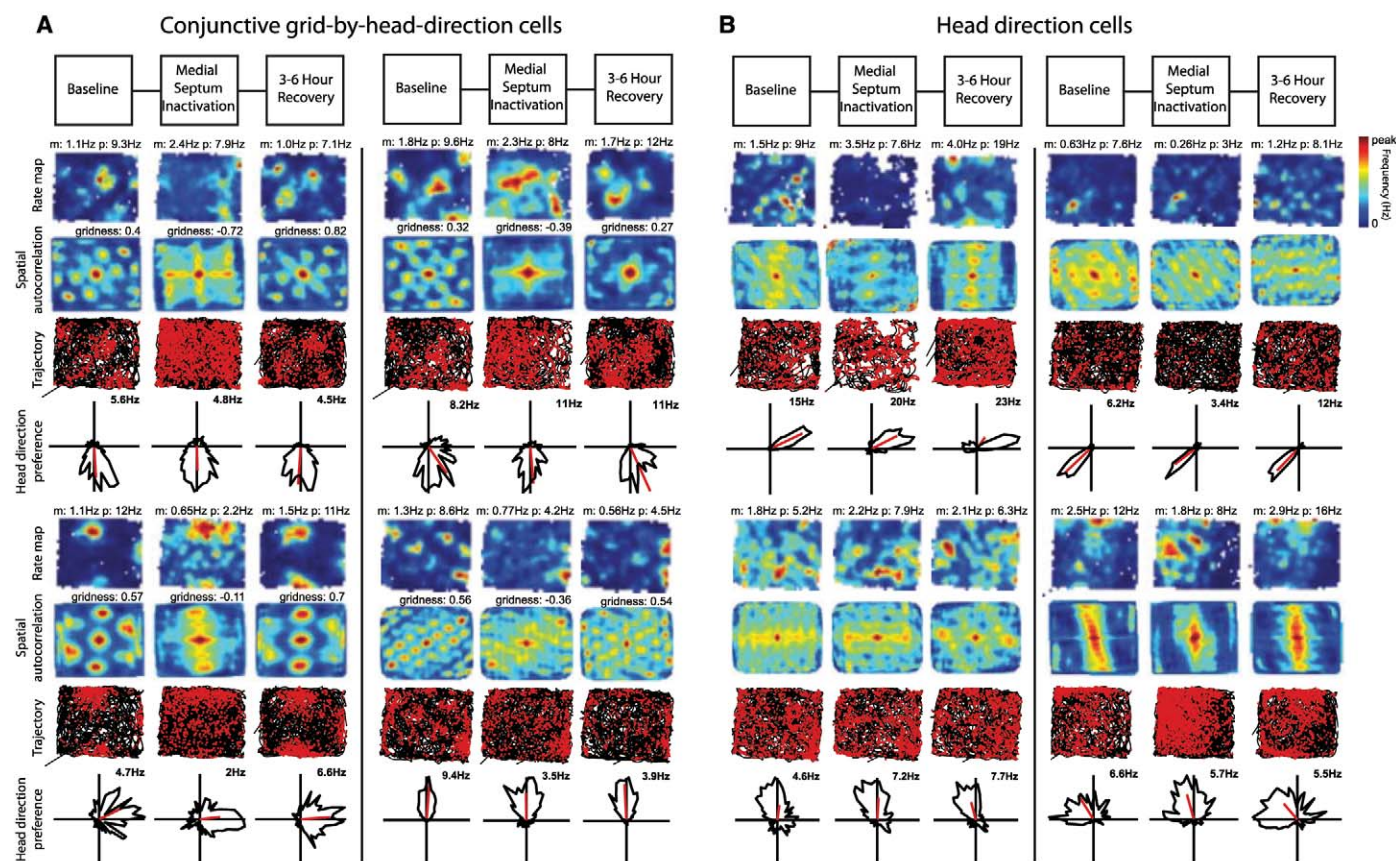
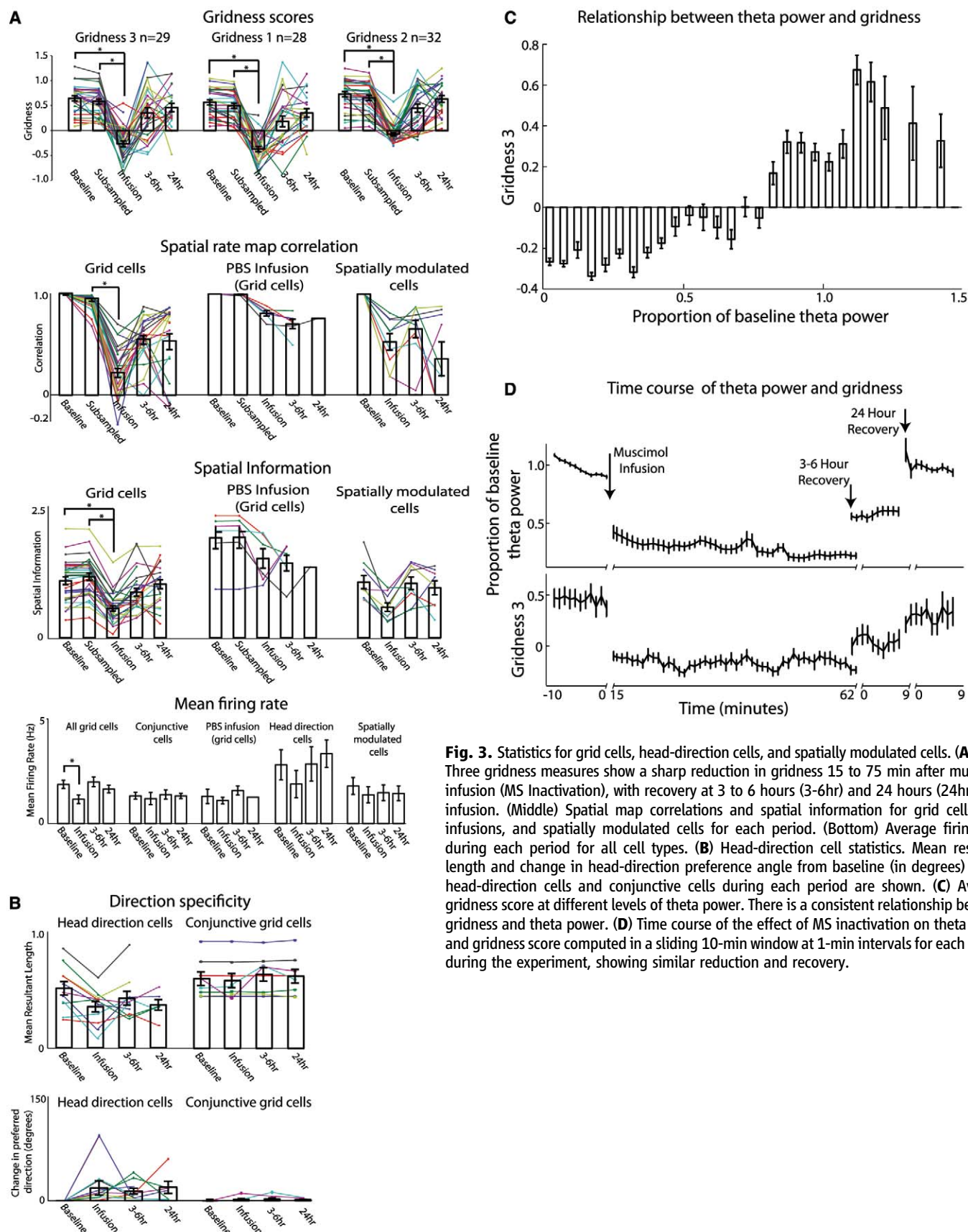


Fig. 2. Head-direction selectivity is spared. (A) Examples of four conjunctive grid-by-head-direction cells. The selectivity for head direction observed before infusion (Baseline) remains during MS inactivation despite the loss of grid cell spatial

periodicity in the spatial maps. (B) Four head-direction-selective cells without spatial periodicity before infusion (Baseline) maintain head-direction preference during MS inactivation. Overall firing rates are not reduced in either cell type.



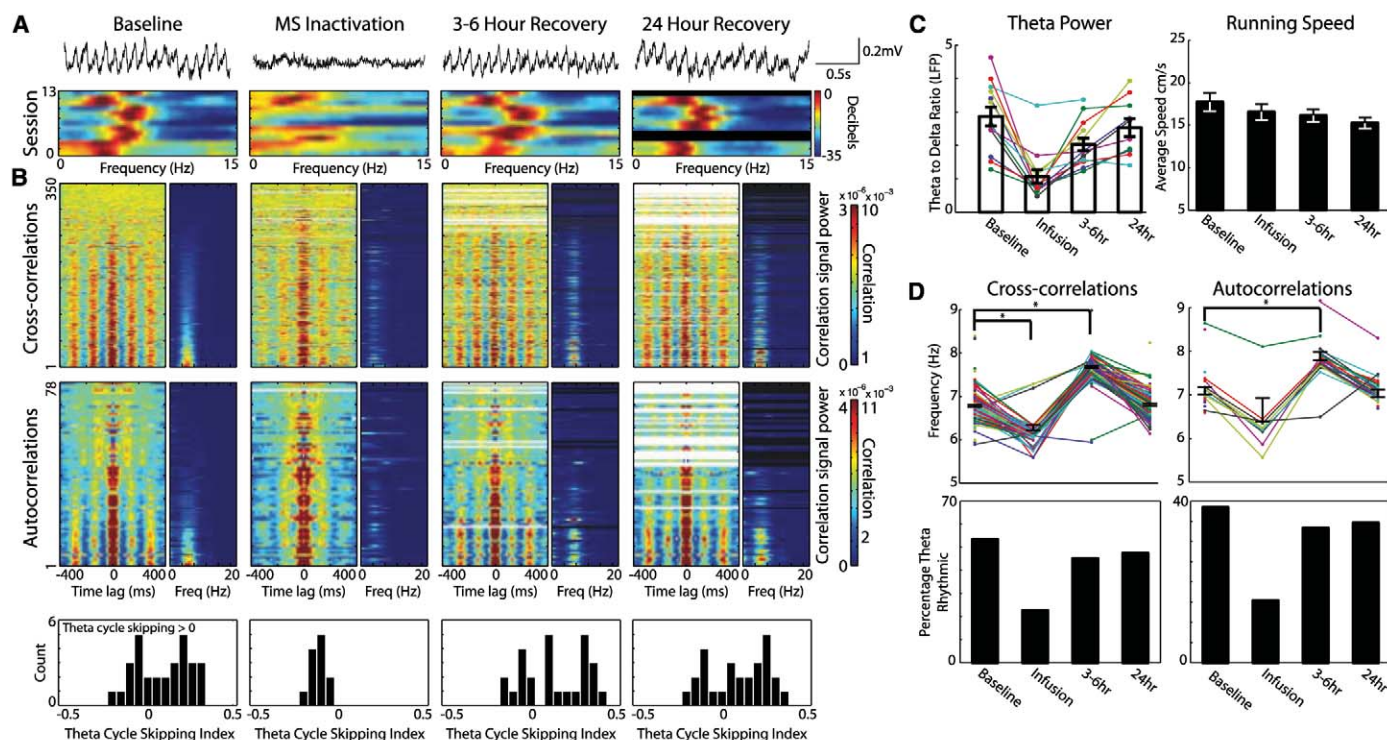


Fig. 4. Temporal coordination of local field potential and spike timing disrupted during MS inactivation. **(A)** (Top) Raw electroencephalogram shows reduction of theta rhythm during MS inactivation. (Bottom) Power spectrum of MEC local field potential for 13 sessions during periods before (Baseline), 15 to 75 min after (MS inactivation), 3 to 6 hours after, and 24 hours after infusion. **(B)** (Top) For each period, the left graph shows temporal cross-correlations ordered by the ratio $\text{Power}_{\text{theta}}/\text{Power}_{\text{signal}}$. The right graph shows respective power spectra. Reduction in theta power after infusion in cross-correlations suggests loss of timing relationships between cells. There is a decrease in frequency during MS inactivation. (Middle) Temporal autocorrelations of spiking show reduction in theta power and frequency during MS

inactivation. (Bottom) Theta cycle skipping index for all theta rhythmic neurons for each period. An index greater than zero indicates that the second peak in autocorrelation is larger than the first peak. Theta skipping is lost during MS inactivation. **(C)** (Left) Theta power (the ratio between power in the theta and delta bands) decreases during MS inactivation. (Right) Average running speed for each period did not change during infusion. **(D)** (Top) Cross-correlation and autocorrelation frequency for each cell pair or cell during each period shows a reduction in frequency during MS inactivation and an increase 3 to 6 hours after infusion. (Bottom) Percentage of cells showing theta rhythmicity in cross-correlations and autocorrelations during each period. There is a reduction of theta rhythmic cells and cell pairs during MS inactivation.

simultaneously recorded neurons during MS inactivation. The combined data suggest that grid cell spatial periodicity is not essential for place cell responses in familiar environments, which is consistent with studies suggesting that the development of place cell responses does not depend on the development of grid cell responses (27, 28).

These data support the hypothesized role of theta rhythm oscillations in the generation of grid cell spatial periodicity (10–12) or at least a role of MS input. The loss of grid cell spatial periodicity could contribute to the spatial memory impairments caused by lesions (3, 4, 18, 29) or inactivation (1, 2, 17) of the MS. These data support a role of neuronal oscillations in the coding of spatial information.

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Trans-Endocytosis of CD80 and CD86: A Molecular Basis for the Cell-Extrinsic Function of CTLA-4

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Cytotoxic T lymphocyte antigen 4 (CTLA-4) is an essential negative regulator of T cell immune responses whose mechanism of action is the subject of debate. CTLA-4 shares two ligands (CD80 and CD86) with a stimulatory receptor, CD28. Here, we show that CTLA-4 can capture its ligands from opposing cells by a process of trans-endocytosis. After removal, these costimulatory ligands are degraded inside CTLA-4-expressing cells, resulting in impaired costimulation via CD28. Acquisition of CD86 from antigen-presenting cells is stimulated by T cell receptor engagement and observed *in vitro* and *in vivo*. These data reveal a mechanism of immune regulation in which CTLA-4 acts as an effector molecule to inhibit CD28 costimulation by the cell-extrinsic depletion of ligands, accounting for many of the known features of the CD28–CTLA-4 system.

The T cell protein cytotoxic T lymphocyte antigen 4 (CTLA-4) is essential to the prevention of autoimmune disease (1–3). Although the molecular basis for CTLA-4 action has been suggested to be a cell-intrinsic inhibitory signal (4) possibly mediated by the cytoplasmic domain (5), a cell-extrinsic function for CTLA-4 is clearly evident from *in vivo* models (6–13). Therefore, a molecular explanation of CTLA-4 function compatible with such a cell-extrinsic mechanism is needed. The intercellular transfer of a ligand from one cell to its receptor on a different cell is observed in both immune settings and elsewhere (14–19). Because of its highly endocytic behavior, we tested whether CTLA-4 could potentially act in such a manner in order to deplete its ligands and extrinsically inhibit T cell activation via CD28. We cultured CTLA-4-expressing (CTLA-4⁺) CHO cells with donor CHO cells expressing a C-terminally tagged CD86 protein (CD86-GFP). Using a flow cytometric assay, we observed substantial transfer of CD86 into CTLA-4⁺ cells (Fig. 1A and fig. S1). This finding was confirmed by using confocal microscopy, in which acquisition of ligand by CTLA-4 caused the appearance of CD86-containing vesicles within the CTLA-4⁺ cell (Fig. 1B). Treatment with the lysosomal inhibitor bafilomycin A caused an increase in detectable CD86–green fluorescent protein (GFP) after transfer (Fig. 1A), which indicated degradation of the transferred ligand inside the CTLA-4⁺ cell. Accordingly, CD86 did not appear on the cell surface of the recipient CTLA-4⁺ cell (fig.

S2). Bafilomycin A treatment did not result in an increase in CTLA-4 expression, which suggested that although CTLA-4 could capture and deliver ligand for degradation, CTLA-4 itself was not degraded (fig. S3). Overall, this process appeared different to the generalized intercellular exchange or “trogocytosis” reported for other receptors (20–22), in which transferred proteins are detected at the cell surface.

To investigate the time course of CD86 acquisition, we performed live-cell imaging of CTLA-4⁺ CHO cells interacting with CHO cells expressing CD86-GFP (fig. S4A and movies S1 and S2) (23). Within minutes of cell contact, we observed a marked concentration of CD86-GFP at the site of cell-cell contact, from which CD86-positive vesicles emanated into the CTLA-4-expressing cell (movies S1 to S4). Quantitation of this process revealed a substantial depletion of GFP fluorescence from the plasma membrane of the CD86 donor cell and a corresponding increase in GFP inside the CTLA-4⁺ recipient cell (fig. S4B). Kinetic analysis by means of flow cytometry also revealed that over 50% of CTLA-4⁺ cells acquired CD80 or CD86 within 3 hours (fig. S5). Furthermore, we estimated the number of CTLA-4 molecules and CD86 molecules expressed by our cell lines to determine the stoichiometry of CD86 depletion (fig. S6). This showed that a ratio of approximately 1:8 (CTLA-4:CD86) molecules was sufficient for functionally relevant depletion.

To confirm that trans-endocytosis of ligand was not an artifact of using GFP-fusion proteins, we also performed experiments with wild-type CD86 expressed in CHO cells. CHO-CD86 cells were cultured alone or with CHO-CTLA-4 cells and then stained for CD86 and CTLA-4 by using antibodies (Fig. 1C). In the absence of CTLA-4, CD86-expressing cells display a characteristic plasma membrane-staining pattern (fig. S7A); however, in the presence of CTLA-4, CD86-containing

vesicles were observed inside CTLA-4 recipient cells (Fig. 1C and fig. S7A). Moreover, because CD86 was stained by using an antibody against the cytoplasmic domain this indicated that the whole ligand had been transferred into the CTLA-4⁺ cell. Analysis of immunofluorescence images showed no acquisition of CTLA-4 by the CD86-expressing cells (fig. S7B). In addition, studies using the membrane dye PKH26 revealed that trans-endocytosis was associated with transfer of small amounts of membrane lipids (fig. S7C). Taken together, these data indicated that protein transfer was unidirectional and appeared to involve transfer of membrane lipid. Because the C terminus of CTLA-4 is required for endocytosis (fig. S8) (24, 25) and shows a remarkable degree of evolutionary conservation, we tested the contribution of this region to CD86 trans-endocytosis by deleting the C terminus of CTLA-4 (CTLA-4 del36). Although CD86 still localized to regions of cell-cell contact (Fig. 1D), wild-type CTLA-4 was much more effective at depleting CD86 than was CTLA-4 del36 (Fig. 1E). As expected, when the CD86-depleted cells from these experiments were used to stimulate T cells, those exposed to wild-type CTLA-4 had impaired stimulatory capacity as compared with those exposed to non-endocytic CTLA-4 (Fig. 1F). Together, these results indicate that by a process of trans-endocytosis, CTLA-4 removes CD86 from neighboring cells, resulting in impaired T cell responses.

To test these observations in a more physiological setting, we activated human CD4⁺CD25[−] T cells in the presence of monocyte-derived dendritic cells (DCs) so as to allow the induction of CTLA-4 and looked for evidence of trans-endocytosis. In the absence of T cells, CD86 was robustly expressed on the surface of DCs (Fig. 2A). In the presence of activated T cells, however, CD86 on the plasma membrane of DCs was reduced and instead found in a punctate pattern that colocalized with CTLA-4 (Fig. 2B). Incubation with a blocking antibody to CTLA-4 prevented the down-regulation of CD86 on the DC as well as the appearance of CD86 in CTLA-4⁺ vesicles in the T cell (Fig. 2, B and C, and fig. S9). Although CD86 was down-regulated in a CTLA-4-dependent manner, expression of other molecules on the DC such as CD40 was unaffected (fig. S9). These results indicated that CTLA-4-mediated trans-endocytosis was specific to CD80 and CD86. To establish whether CTLA-4 was sufficient to confer this function to T cells, we also generated a Jurkat cell line expressing CTLA-4 (fig. S10A). Jurkat cells transduced with CTLA-4 acquired the ability to capture CD86-GFP (fig. S10B). Analysis by means of electron microscopy also showed acquired CD86 in distinct intracellular vesicles within CTLA-4⁺ Jurkat cells (fig. S10C). The use of a number of blocking reagents, including CTLA-4-immunoglobulin and antibody to CD28, confirmed the specificity of CD86-GFP transfer to Jurkat cells (fig. S11 and fig. S12) and demonstrated that

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CD28 was not capable of trans-endocytosis. We have previously shown that transfection of resting human T cells with CTLA-4 confers suppressive activity (8). We therefore tested whether this approach also conferred the ability to capture CD86 from antigen-presenting cells (APCs). CTLA-4-transfected (but not mock-transfected) resting T cells exhibited specific sequestration and internalization of CD86, from the DC (Fig. 2,

D and E), but had no effect on human leukocyte antigen (HLA)-DR expression. Taken together, these data demonstrated CTLA-4 expression by T cells was sufficient to confer the ability to remove CD86 from DCs.

Because T cell receptor (TCR) engagement leads to enhanced trafficking of CTLA-4 to and from the plasma membrane (fig. S13, A and B) (26–28), we predicted that TCR stimulation

should enhance CD86 acquisition. To test this, human CTLA-4⁺ T cell blasts were incubated in the presence of CD86-GFP-expressing CHO cells with or without antibody to CD3. TCR stimulation increased the acquisition of CD86-GFP in a manner that was blocked by antibody to CTLA-4 and enhanced by bafilomycin (Fig. 3A). Similarly, staphylococcal enterotoxin B (SEB)-reactive T cell blasts incubated with SEB-pulsed DCs also showed enhanced acquisition of CD86 (Fig. 3, B and C).

We next determined whether CD4⁺CD25⁺ regulatory T cells (T_{reg} cells) could acquire CD86 from APCs because CTLA-4 is constitutively expressed on T_{reg} cells and is recycled upon stimulation (fig. S13, C and D). DCs were incubated overnight with purified human CD4⁺CD25⁺ T cells or CD4⁺CD25⁺ T_{reg} cells and antibody to CD3. In the presence of CTLA-4⁺ T_{reg} cells, CD86 was down-regulated from the APC surface and observed in intracellular puncta inside the T_{reg} cells. In contrast, CD86 remained on the plasma membrane of DC in the presence of CD4⁺CD25⁺ T cells that lacked CTLA-4 (Fig. 3, D and E). To test whether down-regulation of CD86 by T_{reg} cells affected T cell stimulation, we stimulated T_{reg} cells with DCs in the presence or absence of a blocking antibody to CTLA-4. We then repurified these DCs and used them to stimulate carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled responder T cells. This revealed that blocking CTLA-4 on T_{reg} cells maintained the stimulatory capacity of the DC as compared with where CTLA-4 was available to deplete ligands (fig. S14 A). Moreover, suppression could be overcome by restoring co-stimulation by using CD86-expressing transfectants (fig. S14 B). We also observed that T cell blasts could act as suppressor cells in a CTLA-4-dependent manner (fig. S14 C), again indicating that depletion of co-stimulatory molecules by CTLA-4 has functional consequences.

Our data suggest a model in which antigen stimulation of either T cells or T_{reg} cells promotes the removal and degradation of CD80 and CD86 from APCs by CTLA-4. To test whether this process could also take place in vivo, we generated a system to study trans-endocytosis in mice. We first established that mouse CD4⁺ T cells stimulated in vitro could acquire CD86-GFP from CHO cell targets (fig. S15, A and B). We next developed an in vivo protocol (fig. S16) in which DO11.10 TCR transgenic T cells [specific for a peptide fragment of chicken ovalbumin (OVA) presented by I-A^d] were transferred into Balbc *Rag2*^{-/-} mice that 3 weeks prior had been reconstituted with CD86-GFP-transduced *Rag2*^{-/-} bone marrow. Recipient mice therefore lacked lymphocytes, except the adoptively transferred DO11.10 T cells, and expressed CD86-GFP on their APCs. Seven days after OVA immunization, mice were rechallenged with OVA peptide in the presence of the lysosomal inhibitor chloroquine. T cells were then harvested and immediately analyzed with confocal imaging. This

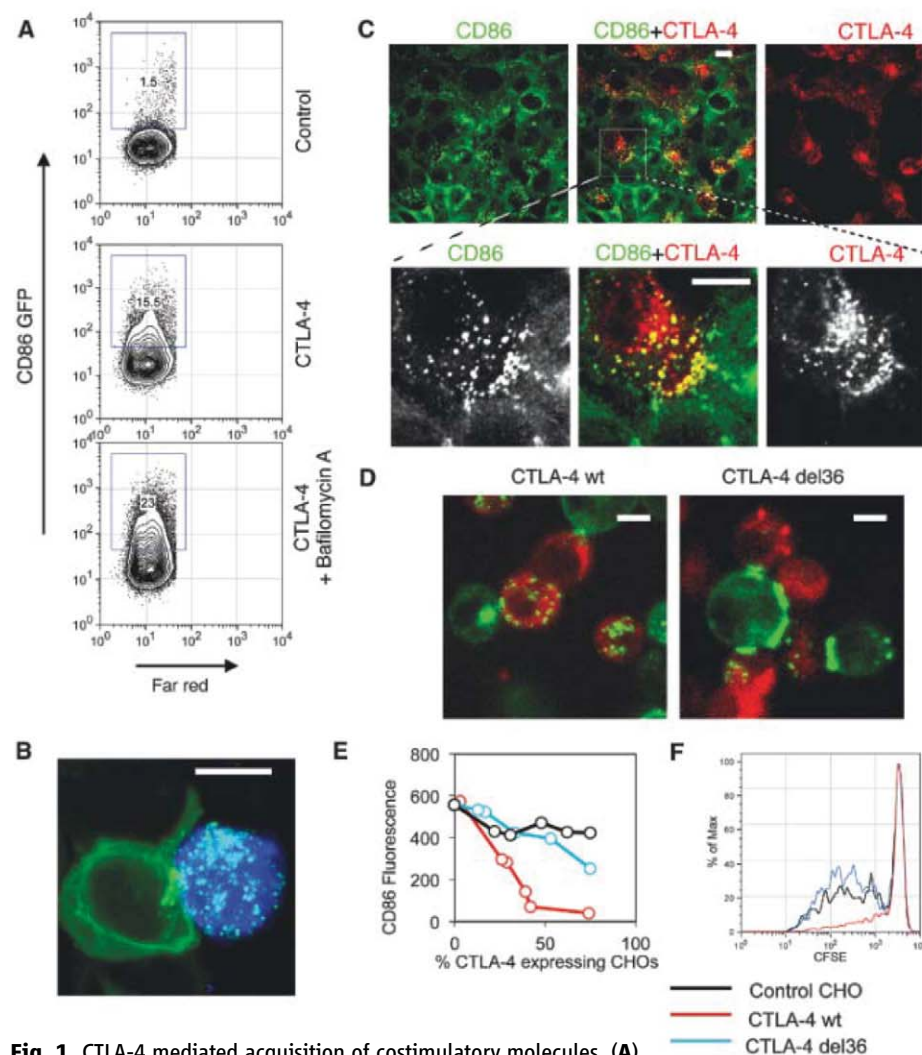


Fig. 1. CTLA-4 mediated acquisition of costimulatory molecules. (A) Flow cytometric analysis of CD86-GFP transfer into CTLA-4-expressing cells. CHO cells expressing CD86-GFP (Far Red-labeled) were cocultured with CHO controls or with CTLA-4⁺ CHO cells in the presence or absence of 10 nM Bafilomycin A (BafA). Singlet CTLA-4-expressing cells were analyzed for GFP acquisition by excluding Far Red⁺ donor cells from analysis (fig. S1). (B) Projection of a confocal z-stack showing a (blue) CTLA-4⁺ CHO cell in contact with a (green) CD86-GFP-expressing CHO cell in the presence of BafA. GFP inside the CTLA-4 cell appears as light blue puncta. (C) Confocal micrographs of adherent CD86-expressing CHO cells and CTLA-4⁺ CHO cells after overnight incubation. CD86 (green) and CTLA-4 (red) were detected through antibody staining. Colocalization of CD86 and CTLA-4 is shown in yellow. (Bottom) An enlargement of the boxed area, with single-color images shown in white for equal contrast. CHO-CD86 cultured alone are shown in fig. S7A. (D) Confocal images of CTLA-4 transfectants (red) expressing wild-type (wt) CTLA-4 or CTLA-4 lacking the cytoplasmic domain (del36) incubated for 2 hours with CD86-GFP-expressing cells (green). (E) Flow cytometric analysis of CD86 surface expression on CHO-CD86 cells co-incubated with increasing numbers of untransfected (control), wild-type CTLA-4, or CTLA-4 del36 cells (expressed as percent of CTLA-4⁺ cells in the coculture). Surface CD86 was detected through antibody staining. (F) Response of CFSE-labeled CD4⁺CD25⁺ T cells stimulated in the presence of antibody to CD3 with CD86-expressing cells fixed after coculture with wild-type CTLA-4 or CTLA-4 del36. All data are single representatives of three or more independent experiments. Scale bars, 10 μ m.

Fig. 2. Human T cells use CTLA-4 to remove CD86 from DCs. (A) Typical CD86 expression on a human monocyte-derived DC cultured in the absence of T cells. (B) DC cultured for 72 hours with antibody-to-CD3-activated $CD4^+CD25^-$ T cells (outlined in white) stained with (green) antibody to CD86 and (red) antibody to CTLA-4. Single-color staining is shown as white for equal contrast. Cells were cocultured in the absence or presence of blocking antibody to CTLA-4. (C) Quantitation of surface CD80 and CD86 expression on DCs after coculture with T cells in the presence or absence of antibody to CTLA-4 determined by means of flow cytometry. Data show mean fluorescence intensity (MFI) change pooled from greater than five experiments with SEM. (D) Confocal micrographs of allogeneic DCs cocultured overnight with CTLA-4-transfected (CTLA-4 TF) or control resting $CD4^+CD25^-$ human T cells. Cultures were fixed and stained with (green) antibody to CD86 or (red) antibody to HLA-DR. White arrowheads highlight position of T cells, and green arrowheads highlight DCs. White images show single-color staining for contrast. (E) MFI of surface CD86 on DCs after incubation with either CTLA-4-transfected or control T cells as determined by means of flow cytometry. All data are representative of at least three independent experiments. Error bars represent the SEM. Scale bars, 10 μ M.

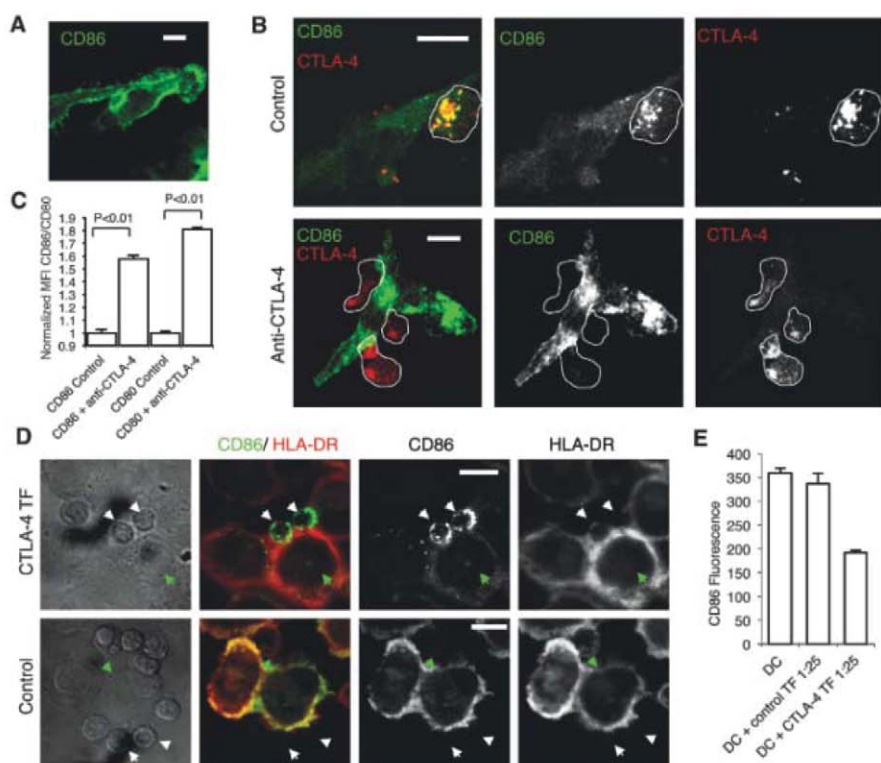
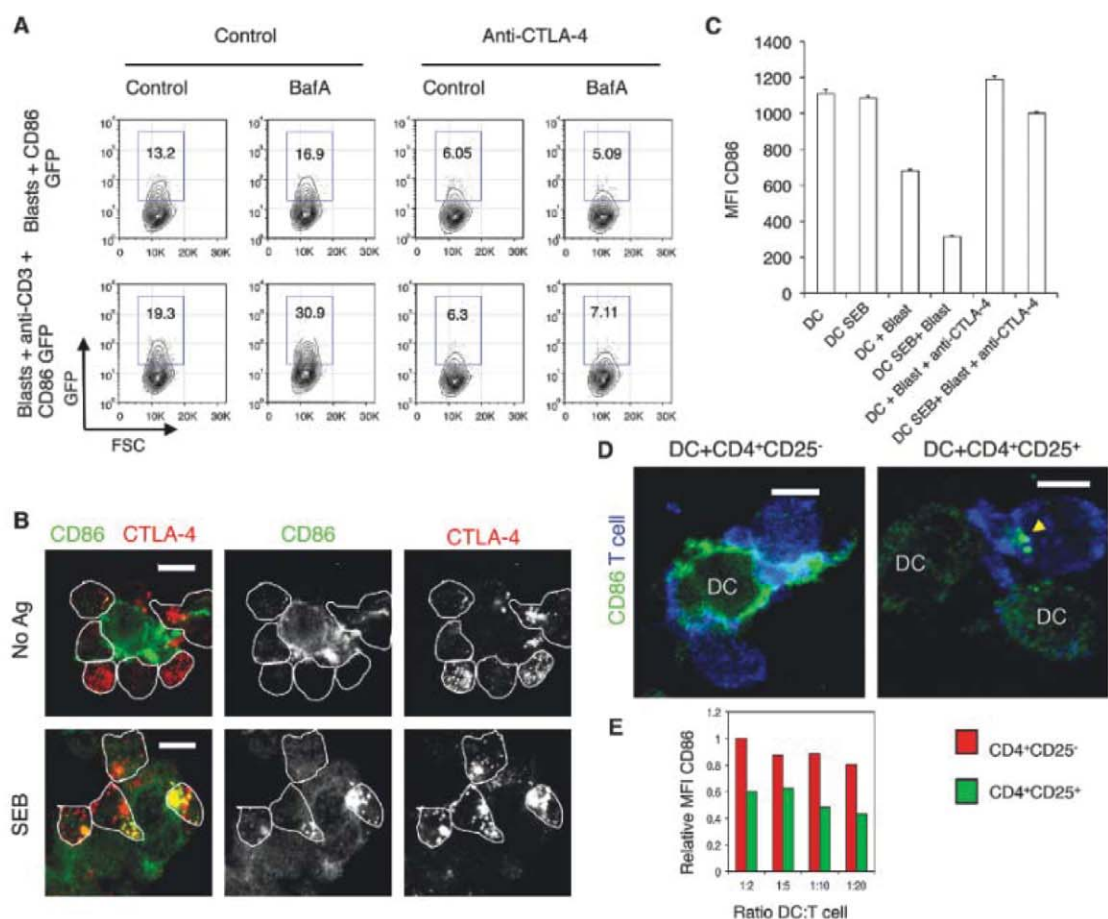


Fig. 3. TCR stimulation promotes CTLA-4 trafficking and trans-endocytosis of CD86. (A) Acquisition of CD86-GFP from CHO cells by human $CD4^+$ T cell blasts in the presence or absence of antibody-to-CD3 stimulation. (Right) The effect of antibody to CTLA-4 on GFP uptake. (B) SEB-specific $CD4^+$ T cell blasts were incubated with either unpulsed or SEB-pulsed DCs. Cells were fixed and stained with (green) antibody to CD86 and (red) antibody to CTLA-4. Yellow indicates colocalization. Single colors are shown in white for equal contrast. (C) Surface levels of CD86 on DCs incubated with SEB-specific T cell blasts for 16 hours as determined by means of flow cytometry. (D) $CD4^+CD25^+$ (T_{reg}) cells or $CD4^+CD25^-$ T cells were incubated with DCs and antibody to CD3 overnight, fixed, stained with (green) antibody to CD86 and (blue) antibody to CD3 for T cells, and visualized with confocal microscopy. The yellow arrow indicates CD86 puncta within T cells. (E) Surface levels of CD86 on DCs incubated with $CD4^+CD25^+$ (T_{reg}) cells and $CD4^+CD25^-$ T cells overnight determined by means of flow cytometry. All data are representative of at least three independent experiments. Error bars represent the SEM. Scale bars, 10 μ M.



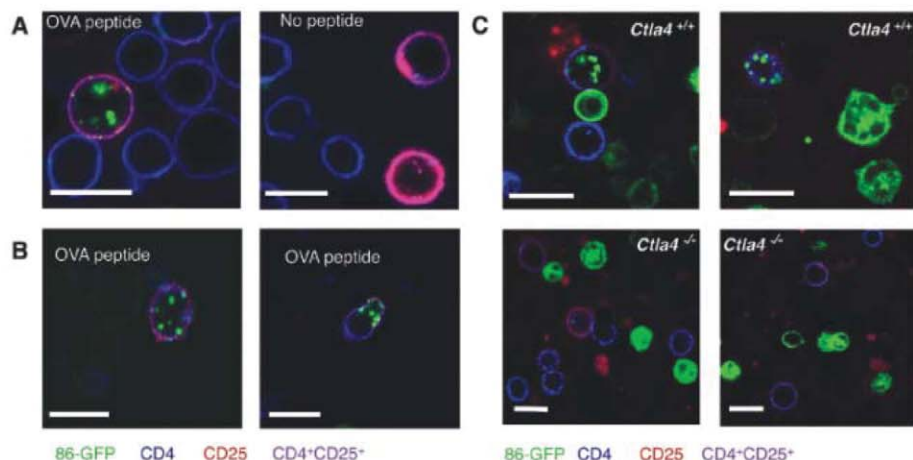


Fig. 4. In vivo capture of CD86 by CTLA-4. Balbc *Rag2*^{-/-} mice were reconstituted with CD86-GFP transduced Balbc *Rag2*^{-/-} bone marrow to permit the development of APCs expressing CD86-GFP. Three weeks later, mice were injected with DO11.10 CD4⁺ T cells and immunized as described in fig. S16. (A) Six hours after intravenous OVA peptide challenge, splenocytes were harvested, labeled at 4°C for (blue) CD4 and (red) CD25, and immediately imaged with confocal microscopy. Representative images of T cells from OVA peptide challenged or unchallenged mice are shown. (B) Representative images of CD4⁺ T cells purified from spleen after treatment in vivo with peptide showing CD4 and CD25 staining. (C) CD4⁺ T cells from either *Ctla4*^{+/+} or *Ctla4*^{-/-} (*Rag2*^{-/-} DO11.10 Rip-mOVA) mice were injected into mice that previously received CD86-GFP *Rag2*^{-/-} bone marrow cells. Cells were rechallenged with OVA in vivo as above. Splenocytes were isolated and immediately analyzed for (green) CD86-GFP, (red) CD25, and (blue) CD4 by use of confocal microscopy. Two representative panels are shown for both *Ctla4*^{+/+} and *Ctla4*^{-/-} conditions. Data are representative of three independent experiments. Scale bars, 10 μm.

revealed CD86-GFP in endosomal compartments of antigen-stimulated, but not unstimulated, T cells (Fig. 4A). Moreover, internalized CD86-GFP was restricted to CD4⁺CD25⁺ T cells (Fig. 4B), which is consistent with the expression of CTLA-4 by these cells. These cells did not express Foxp3 (fig. S17A, left). The amount of CD86-GFP transfer was extensive because the mean GFP fluorescence inside T cells approached the amounts on donor cells themselves (fig. S17B). To establish the requirement for CTLA-4, we tested CD4⁺ cells from *Ctla4*^{+/+} or *Ctla4*^{-/-}, *Rag2*^{-/-} DO11.10 mice bred to mice that express OVA under the control of the rat insulin promoter (Rip-mOVA). These mice were useful because they develop OVA-specific T_{reg} cells, and we have shown that those deficient in CTLA-4 fail to regulate diabetes (13). After in vivo rechallenge with OVA peptide, CD86-GFP acquisition was only observed in CD4⁺CD25⁺ T cells capable of CTLA-4 expression but not those from *Ctla4*^{-/-} mice (Fig. 4C). Moreover, CD4⁺CD25⁺ T cells from these mice were almost entirely Foxp3⁺ (fig. S17A, right). Overall, analysis indicated that approximately 25 to 40% of wild-type CD4⁺CD25⁺ T cells acquired ligand (fig. S17C). No transfer of control (unfused) GFP molecules was observed (fig. S17D). Together, these data indicate that both Foxp3⁺ and Foxp3⁻ T cells are capable of CD86 acquisition in vivo.

The CTLA-4 molecule plays a critical role in suppressing autoimmunity and maintaining immune homeostasis; however, its precise mechanism of action has been a subject of debate.

Recent data have provided evidence that CTLA-4 can perform a nonredundant effector function for T_{reg} cells, requiring a cell-extrinsic mechanism of action (9, 13). We demonstrated a cell-extrinsic model of CTLA-4 function that operates by the removal of costimulatory ligands from APCs via trans-endocytosis. Using both human and mouse T cells, we established that trans-endocytosis of ligand occurs in precisely the same settings in which we have demonstrated CTLA-4-dependent regulation (8, 13). Moreover, this mechanism is specific for CTLA-4/CD28 ligands and operates in an antigen-dependent manner in vivo. Our data are also compatible with several studies that demonstrate reduced levels of costimulatory ligand expression in the presence of CTLA-4-expressing T_{reg} cells (9, 29, 30) and are consistent with a role for CTLA-4 on both activated and regulatory T cells (9, 31, 32). Taken together, these results not only provide a widely applicable basis for CTLA-4 function but also offer cogent explanations for long-standing paradoxes in the field: namely, how CTLA-4 can function in a cell-extrinsic manner, why CTLA-4 shares ligands with the stimulatory receptor CD28, and why CTLA-4 exhibits endocytic behavior. Although not excluding other mechanisms of CTLA-4 action, we suggest that CTLA-4 carries out the same molecular functions, whether expressed by T cells or by T_{reg} cells—a concept that has considerable implications for our understanding of

immune homeostasis. Together, these data provide a new framework for studies of CTLA-4 and should facilitate our understanding of its immunoregulatory role in the key settings of cancer, HIV infection, and autoimmune disease.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1202947/DC1
Materials and Methods
Figs. S1 to S17
References 33 and 34
Movies S1 to S4

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A Radically Different Mechanism for S-Adenosylmethionine–Dependent Methyltransferases

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Methylation of small molecules and macromolecules is crucial in metabolism, cell signaling, and epigenetic programming and is most often achieved by S-adenosylmethionine (SAM)–dependent methyltransferases. Most employ an S_N2 mechanism to methylate nucleophilic sites on their substrates, but recently, radical SAM enzymes have been identified that methylate carbon atoms that are not inherently nucleophilic via the intermediacy of a 5'-deoxyadenosyl 5'-radical. We have determined the mechanisms of two such reactions targeting the sp^2 -hybridized carbons at positions 2 and 8 of adenosine 2503 in 23S ribosomal RNA, catalyzed by RlmN and Cfr, respectively. In neither case is a methyl group transferred directly from SAM to the RNA; rather, both reactions proceed by a ping-pong mechanism involving intermediate methylation of a conserved cysteine residue.

A number of different posttranscriptional and posttranslational modifications adorn protein and RNA components of the ribosome. Although the functions of most of these modifications are currently unknown, many are believed to confer stability and translational fidelity and to direct proper ribosomal assembly (1–3). Among the most common modifications is a methyl group derived from S-adenosylmethionine (SAM) that is appended to specific atoms in amino acid side chains or nucleotide bases or sugars. Most of these groups are added by S_N2 displacement mechanisms, involving attack of a nucleophile on the methyl group of SAM with concomitant release of S-adenosyl-L-homocysteine (4, 5). Intriguingly, two SAM-dependent methylations of 23S ribosomal RNA (rRNA) of bacterial ribosomes involve electrophilic rather than nucleophilic sp^2 -hybridized carbons (Fig. 1) (6–8); the enzymes RlmN (YfgB) and Cfr methylate C-2 and C-8 of adenosine 2503 (A2503), respectively. Moreover, the poor acidity of the protons attached to C-2 and C-8 precludes their removal by typical polar (acid/base) processes that are mediated by the side chains of the 20 common amino acids (9, 10).

About half of all antibiotics currently in use target the bacterial ribosome, with most binding to sites on the large (50S) subunit, disrupting functions associated with guanosine 5'-triphosphate hydrolysis, peptide-bond formation, and exit of the nascent polypeptide (1, 11). The latter two functions are inhibited by several classes of antibiotics, which bind to overlapping regions. Thus, bacterial interventions to resist an antibiotic that

targets this region often result in resistance to multiple drugs (1, 12). A2503 of 23S rRNA is in the peptidyltransferase center of the 50S subunit near the entrance to the exit tunnel for the nascent peptide (13–16). Modification of C-2 is thought to play a housekeeping function (6, 17). Although the *yfgB* gene is not essential, *Escherichia coli* *yfgB*-null strains lose to wild-type (WT) strains in cointegration competition experiments (17). Modification of C-8 of A2503 confers resistance to several classes of antibiotics, including phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A (8, 18–20). The *cfr* gene, which encodes this activity, was first identified on a plasmid iso-

lated from *Staphylococcus sciuri*, an animal pathogen (19). More recently, it has been found on the chromosome of a methicillin-resistant strain of *S. aureus* (MRSA) obtained from a hospital isolate along with the *ermB* gene, which encodes a 23S A2058 dimethylase (21). Co-expression of these genes renders the bacterium resistant to all currently used antibiotics that target the 50S subunit (21). Bioinformatics analysis indicates that RlmN is widespread throughout eubacteria and that Cfr evolved directly from it as an antibiotic-resistance mechanism (22). In fact, Cfr, which shares 33% sequence identity with *E. coli* RlmN, also catalyzes methylation at C-2 of A2503, although it is not the preferred target (7).

RlmN and Cfr belong to a class of SAM-dependent enzymes designated radical SAM (RS), which reductively cleave SAM to give a 5'-deoxyadenosyl 5'-radical (5'-dA•) rather than to mediate alkylation reactions (using polar mechanisms) (23). The 5'-dA• intermediate is used to abstract key hydrogen atoms from the substrate; however, the transformations that follow differ widely among RS reactions (24–26). All RS enzymes contain at least one [4Fe-4S] cluster—typically coordinated by the Cys residues of a CxxxCxxC motif—which supplies the electron for the reductive cleavage of SAM. In *E. coli*, the iron-sulfur (Fe/S) cluster is reduced by an electron derived from the flavodoxin/flavodoxin reductase (Flv/Flx) reducing system, whereas in vitro it can also be supplied by low-potential artificial reductants such as dithionite or illuminated deazaflavin (27).

In vitro studies by Yan *et al.* (28) showed that in RlmN and Cfr, SAM acts as both a radical

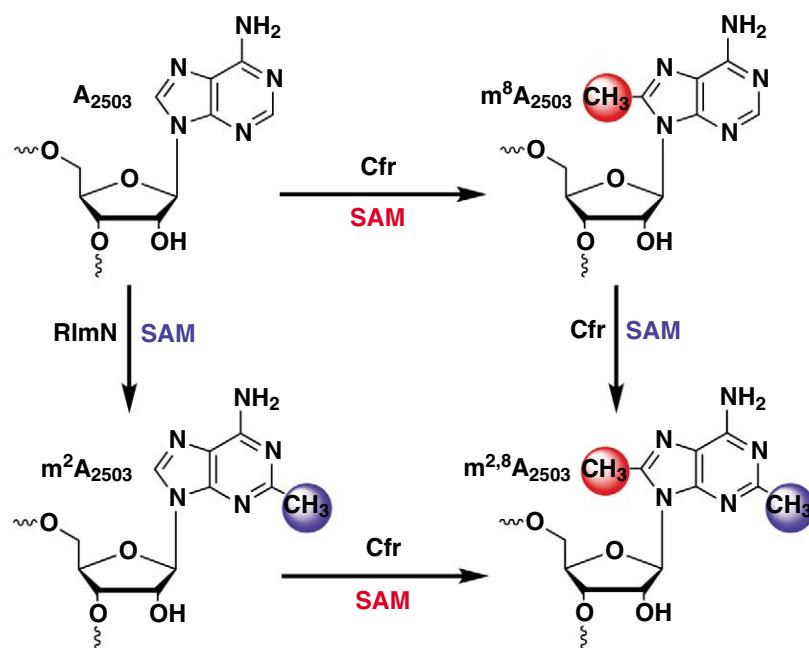


Fig. 1. Reactions catalyzed by RlmN and Cfr. RlmN catalyzes uniquely methylation at C-2, whereas Cfr catalyzes methylation at C-8 and C-2, although C-8 is the preferred target.

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generator and as the source of the appended methyl group. Moreover, these studies showed that both enzymes act preferentially on naked 23S rRNA, of which helices 89 and 90 to 92 in domain V are critical for substrate recognition and turnover (28). To gain further insight into the mechanism of catalysis by RlmN and Cfr, we initiated activity determinations using a seven-nucleotide oligomer (7-mer) spanning positions 2500 to 2506 on rRNA, which included the

naturally occurring pseudouridine modification at position 2504. Figure 2 shows mass spectra of the resulting methylated adenosine (CH₃-Ad) products of a reaction containing 500 μ M WT RlmN (RlmN_{WT}), 1.5 mM SAM, and 250 μ M RNA substrate (29). A chromatographic peak at retention time 6.85 min (Fig. 2, red trace) exhibits a mass spectrometry (MS) peak at mass/charge ratio (m/z) = 282.1 (Fig. 2, inset, red trace) and a fragmentation pattern consistent with that of

methylation at the 2 position of adenosine (m^2A) (7), indicating that the 7-mer can indeed be methylated by RlmN. MS peaks at 283.1 and 284.1 m/z (Fig. 2, inset) derive from natural-abundance ¹³C in the product. Use of the *in vivo* reducing system (Flv/Flx/NADPH, where NADPH is the reduced form of nicotinamide adenine dinucleotide phosphate) in place of dithionite does not give turnover, even after 18 hours of incubation. To confirm that the methyl group derives from SAM, as had been shown previously by Yan *et al.* (28), we incubated both RlmN_{WT} and Cfr as described above, with the exception that SAM was replaced with *S*-adenosyl-L-[methyl-*d*₃] methionine (*d*₃-SAM). Unexpectedly, the RNA product of the RlmN_{WT} reaction gives the same m/z and isotopic distribution (Fig. 2, inset, solid black trace) as the product of the reaction with unlabeled SAM, suggesting that the methyl group transferred under single-turnover conditions (protein in excess of substrate) does not reflect the isotopic composition of SAM added to the assay mixture. To test whether the methyl group could derive from a SAM molecule that bound tightly to the enzyme during its production in *E. coli*, a 200 μ M sample of RlmN_{WT} was quenched in acid and analyzed (29); SAM was not detected (fig. S2). We obtained similar results when Cfr (500 μ M) was incubated with *d*₃-SAM (1.5 mM) and the 7-mer RNA substrate (250 μ M) under single-turnover conditions (Fig. 2, dotted black trace). In this case, the product's retention time is slightly less (6.8 min) than that of the product from the RlmN reaction but identical to that of a synthetic m^8A standard (figs. S1 and S3). In addition, its m/z value and fragmentation pattern are also identical to those of the synthetic m^8A standard and consistent with published results (7). Importantly, the methyl group transferred to the adenosine corresponding to A2503 is again found to lack deuterium from *d*₃-SAM. Thus, the same surprising isotope-tracing result is found for Cfr.

To test the possibility that the methyl group transferred in the first turnover derives from the protein, both RlmN_{WT} and Cfr were produced in and isolated from an *E. coli* methionine auxotroph cultured in the presence of [methyl-*d*₃] methionine (*d*₃-Met) and then used in subsequent activity determinations (30). Figure 3 shows mass spectra of the associated products. As described above, when unlabeled RlmN_{WT} (Fig. 3A) or Cfr (Fig. 3B) is incubated with *d*₃-SAM (black traces), the CH₃-Ad products are found to lack deuterium (m/z 282.1). In contrast, when RlmN_{WT} (Fig. 3A) or Cfr (Fig. 3B) isolated from *E. coli* supplemented with *d*₃-Met during growth [RlmN (*d*₃-Met) and Cfr (*d*₃-Met), respectively] is incubated in the presence of unlabeled SAM (red traces), the CH₃-Ad products exhibit peaks corresponding to m/z = 284.1, two mass units higher. This result implies that the transferred methyl group contains two deuteriums and thus does derive from the protein. The finding that a peak for m/z = 285.1 (corresponding to three deuteriums) is not observed implies that methyl transfer takes

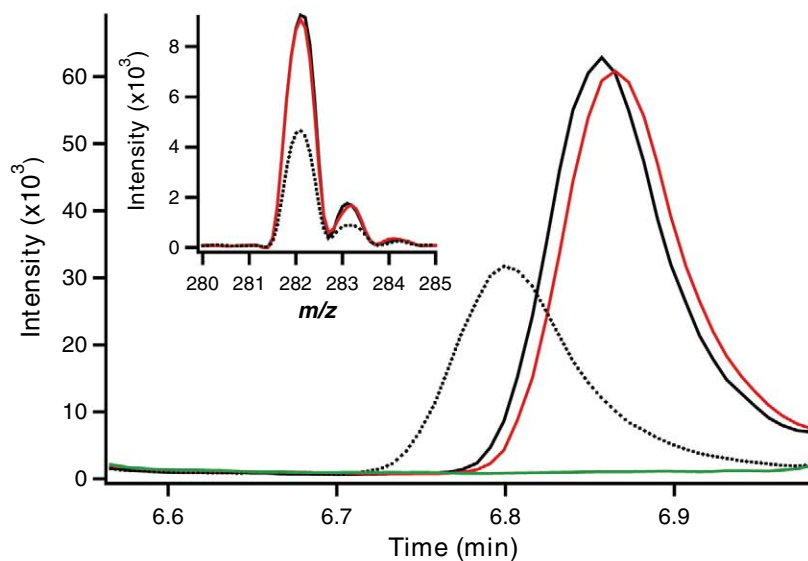


Fig. 2. Liquid chromatography–MS analyses of the methylated products of the RlmN and Cfr reactions. Single-turnover experiment in the presence of 250 μ M 7-mer RNA substrate using 500 μ M RlmN_{WT} and 1 mM SAM (red solid trace), 500 μ M RlmN_{WT} and 1 mM *d*₃-SAM (black solid trace), or 500 μ M Cfr and 1 mM *d*₃-SAM (black dotted trace). The peak at 6.8 min (black dotted trace) corresponds to m^8A , with its associated mass spectrum in the above inset (black dotted trace). The peak at 6.85 min corresponds to m^2A generated in the presence of SAM or *d*₃-SAM with their associated mass spectra in the above inset (red and black solid traces, respectively). The green trace is a control corresponding to the above reactions in the absence of Cfr or RlmN_{WT}.

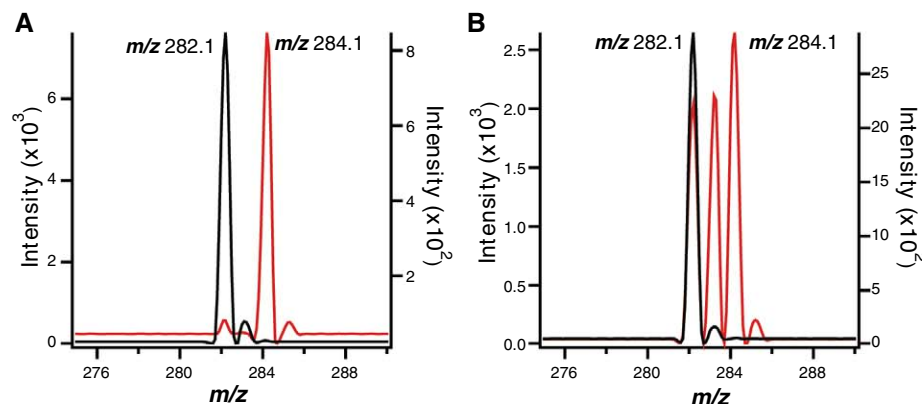


Fig. 3. Mass spectra of CH₃-Ad products derived from RlmN_{WT} and Cfr produced in and isolated from *E. coli* grown in the presence or absence of *d*₃-Met. Each experiment contains 250 μ M 7-mer RNA substrate with (A) 400 μ M RlmN_{WT} and 1 mM *d*₃-SAM (black trace) or 400 μ M RlmN_{WT} (*d*₃-Met) with 1 mM SAM (red trace) or (B) 300 μ M Cfr and 1 mM *d*₃-SAM (black trace) or 300 μ M Cfr (*d*₃-Met) with 1 mM SAM (red trace). Peaks at m/z 282.1 correspond to m^2A (RlmN_{WT}) or m^8A (Cfr) with no deuterium enrichment, whereas peaks at m/z 284.1 correspond to m^2A (RlmN_{WT}) or m^8A (Cfr) with two deuterium atoms. No major peaks at m/z 285.1 are observed, which would correspond to m^2A (RlmN_{WT}) or m^8A (Cfr) with three deuterium atoms.

place with the loss of one deuterium atom. This result stands in contrast to the accepted mechanism of SAM-dependent methyl transfer, in which the methyl group is transferred without exchange of any of its three hydrogens (4, 5). The ladder of m/z values (284.1, 283.1, and 282.1) seen with Cfr (d_3 -Met) (Fig. 3B, red trace) suggests the presence of a step that effects hydrogen exchange with solvent either before or after methyl transfer.

In all RS enzymes characterized to date, SAM is reductively cleaved to a 5'-dA• intermediate. Many RS enzymes catalyze abortive cleavage of SAM in the presence of dithionite, which can complicate efforts to assess the role of the 5'-dA•

(31, 32). As has been shown previously, RlmN_{WT} and Cfr both cleave SAM abortively, even in the absence of substrate (fig. S4) (28, 33). Studies on other RS enzymes have shown that abortive cleavage is reduced substantially—though not always completely (fig. S4)—when dithionite is replaced by the *in vivo* reducing system (Flv/Flx/NADPH) (31, 34, 35). However, as described above, the *in vivo* reducing system does not support turnover of the 7-mer RNA substrate. Studies by Yan *et al.* have shown that a segment of 23S rRNA spanning nucleotides 2018 to 2788 supports turnover at least as well as the entire 23S rRNA (28). Therefore, the corresponding 771-

nucleotide RNA substrate was synthesized by *in vitro* transcription and used in single-turnover activity determinations (29). Figure 4 shows mass spectra of 5'-dA analyzed from reactions conducted with RlmN (d_3 -Met) (Fig. 4A) and Cfr (d_3 -Met) (Fig. 4B) in the presence of unlabeled SAM. The dotted red line in both panels corresponds to a 5'-dA standard at natural abundance, which displays the expected $m/z = 252.1$. The solid black line in both panels corresponds to 5'-dA generated during turnover. Astonishingly, the results demonstrate substantial deuterium enrichment in 5'-dA, implying that the role of the 5'-dA• is not to abstract a hydrogen atom from C-2 or C-8 of the substrate as previously suggested (28), but instead to abstract a hydrogen atom from a protein-bound methyl group (36).

To identify the amino acid that donates a methyl group, we performed MS on tryptic digests of RlmN_{WT} that had been reduced and alkylated with iodoacetamide (29). One peptide (GDDIDAAC³⁵⁵QLAGDVIDR) (37) contained a methylated Cys residue (m/z 909.42017, 45 spectra, 76%) or was alkylated by iodoacetamide (m/z 930.92358, 14 spectra, 24%). The masses of the intact precursors are within 3 parts per million of the theoretical values, and both the *b*- and *y*-ion series produced by fragmentation unambiguously define the site of the methyl modification as Cys³⁵⁵ (fig. S5).

A mechanism for catalysis by RlmN_{WT} that is consistent with observations is proposed in Fig. 5. In the initial, priming step, a methyl group from the first molecule of SAM is transferred to

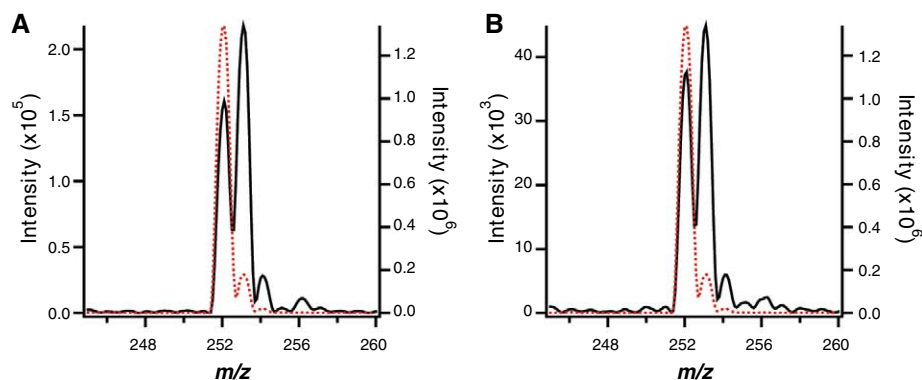


Fig. 4. Analysis of 5'-dA isolated from reactions conducted with RlmN (d_3 -Met) and Cfr (d_3 -Met) in the presence of unlabeled SAM, (A) 150 μ M RlmN (d_3 -Met), and (B) 150 μ M Cfr (d_3 -Met). Both reactions contained 1.5 mM SAM (black trace), 20 μ M 771-mer RNA substrate, and the Flv/Flx reducing system. The red dotted traces correspond to the mass spectrum of commercially available 5'-dA.

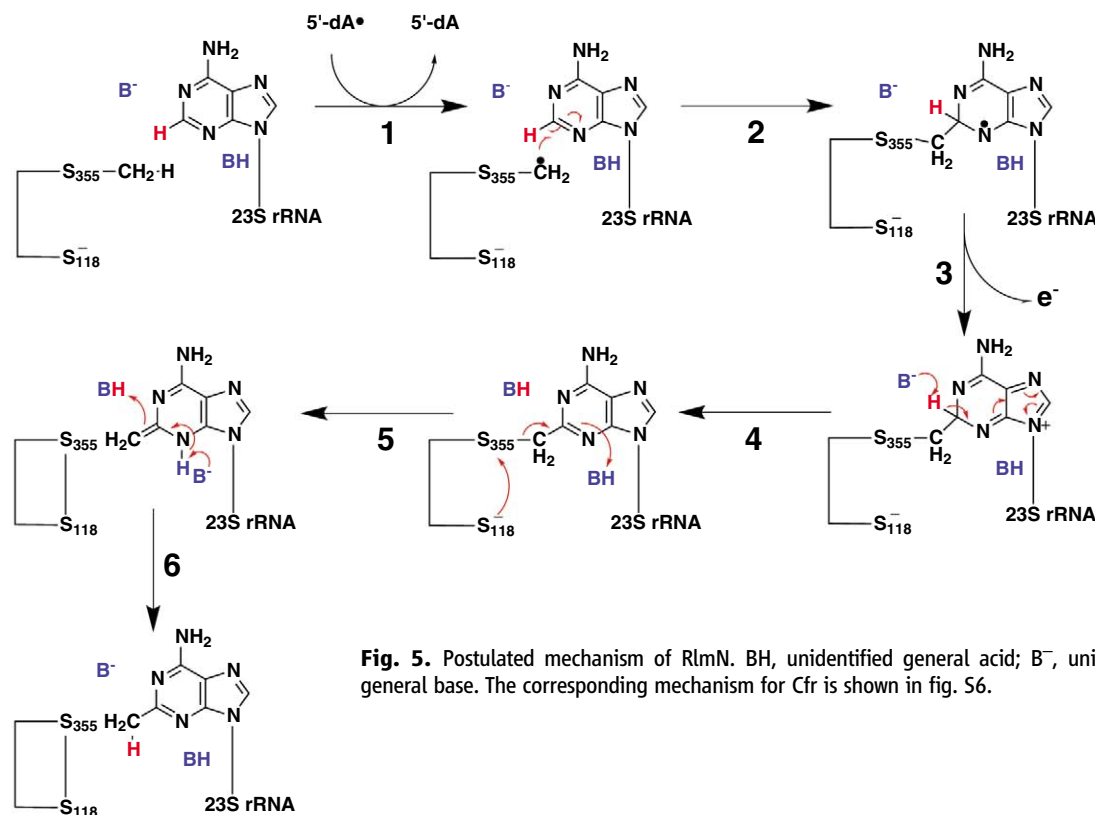


Fig. 5. Postulated mechanism of RlmN. BH, unidentified general acid; B⁻, unidentified general base. The corresponding mechanism for Cfr is shown in fig. S6.

Cys³⁵⁵ by a typical S_N2 displacement mechanism. Reductive cleavage of a second SAM molecule gives the 5'-dA• radical, which, in the subsequent step of the mechanism (1), abstracts a hydrogen atom from the protein-bound methyl group to yield a neutral, carbon-centered radical. Attack of the carbon-centered radical on sp²-hybridized C-2 of A2503 in 23S rRNA results in formation of a carbon-carbon bond and generation of a resonance-delocalized radical on the nucleotide base (2). Loss of an electron (3)—perhaps back to the Fe/S cluster—and abstraction of the proton from C-2 give the alkylated product cross-linked to the protein via Cys³⁵⁵ (4). This cross-link is resolved by attack of the Cys¹¹⁸ thiolate (vide infra) onto Cys³⁵⁵ to yield a disulfide bond and an enamine (5), which collapses to the methylated product upon tautomerization and acquisition of a proton (6). An analogous mechanism for Cfr-catalyzed methylation of C-8 is depicted in fig. S6. The mechanism provides rationale for the exchange of solvent hydrons into the product via reversible imine/enamine tautomerization initiated by cleavage of the thioether linkage upon disulfide formation.

The mechanism also allows rationalization of previously published results that were incompletely understood (22). Both RlmN and Cfr contain five conserved Cys residues, of which three are in the canonical C¹¹²xxxC¹¹⁶xxC¹¹⁹ (*S. aureus* Cfr numbering) RS motif. In vitro mutagenesis studies by Yan *et al.* showed, as expected, that substitution of any of these Cys residues with Ala residues abrogates turnover (28), consistent with in vivo studies by Giessing *et al.* and Kaminska *et al.* (7, 22). Further studies by Kaminska *et al.* showed that the remaining two conserved Cys residues (Cys¹⁰⁵ and Cys³³⁸) are also absolutely required for generation of m⁸A by Cfr. The Cys¹⁰⁵→Ala¹⁰⁵ (C105A) substitution resulted in a stop in a reverse transcription reaction used to assess modification of the RNA substrate, suggesting that some type of modification took place. However, failure of the C105A variant to elicit resistance to florfenicol and tiamulin in vivo suggested that the 23S rRNA did not contain the m⁸A modification (22). RlmN_{C118A} used in our studies displays a maximum wavelength at 265 nm (fig. S7B) rather than the 280 nm that is observed for RlmN_{WT} (fig. S7A), suggesting that it contains bound nucleic acid. Figure S8 depicts an elution profile of RlmN_{C118A} subjected to digestion with P1 nuclease and alkaline phosphatase, which shows not only the canonical RNA bases but also pseudouridine and other modified bases, confirming the presence of RNA. These characteristics are not observed for WT Cfr (fig. S7D), RlmN_{WT} (fig. S7A), or RlmN_{C355A} (fig. S7C), consistent with a model in which Cys¹¹⁸ is required to resolve the covalently bound intermediate.

This strategy of generating a covalent methylcysteinyl intermediate obviates a potential steric clash between the hydrogen-abstracting species and the methyl-donating species, given that both would need to occupy the same space for proper

orbital alignment for transfer to or from an sp²-hybridized carbon. Moreover, there is no precedent in enzymology for abstracting a hydrogen atom from an sp²-hybridized carbon. Furthermore, a Cys residue is an energetically favorable methyl carrier for radical addition, because adjacent sulfur atoms stabilize carbon-centered radicals and therefore decrease the homolytic bond-dissociation energy associated with their generation (38). The ability of Cys residues to form reversible disulfide bonds allows for reductive cleavage of the otherwise stable thioether covalent adduct.

Although the proposed mechanisms of RlmN and Cfr are similar, there are a few differences. Cfr appears to have evolved directly from RlmN using the same mechanistic strategy to methylate C-8 of A2503, but still retaining some ability to methylate C-2. Cfr's bifunctional activity suggests that its substrate-binding pocket is less rigid, allowing for at least two different conformations of A2503 that would put either C-2 or C-8 in resonance with the enzyme's catalytic machinery. This flexibility might be accompanied by access of solvent to the active site, which manifests itself in exchange of solvent-derived hydrons with the product. Or, in the absence of solvent access to the active site, isotope exchange in the case of Cfr might result from a lysyl residue acting as a general acid/base, whereas in the case of RlmN it would be a monoprotonic residue.

A number of other RS enzymes have been predicted to catalyze methyltransfer to unactivated carbon atoms in pathways that involve the biosynthesis of a number of secondary metabolites (39), as well as specific bacteriochlorophylls (40). These enzymes are all annotated as cobalamin-binding proteins, and cobalamin is believed to be the source of the appended methyl group (41, 42). Our study shows how such a reaction might be accomplished without this additional complex cofactor.

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36. The peak at *m/z* = 252.1 in the black traces (RlmN and Cfr reactions) derives predominantly from abortive cleavage of SAM (fig. S4), in which the 5'-dA• abstracts a hydrogen atom that is ultimately solvent-derived, but also from a fraction of the enzyme that is isolated in its unmethylated state and is remethylated by unlabeled SAM.
37. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Supporting Online Material

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Methods
SOM Text
Figs. S1 to S8
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A Crystal Structure of the Complex Between Human Complement Receptor 2 and Its Ligand C3d

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The interaction of complement receptor 2 (CR2)—which is present on B cells and follicular dendritic cells—with its antigen-bound ligand C3d results in an enhanced antibody response, thus providing an important link between the innate and adaptive immune systems. Although a cocrystal structure of a complex between C3d and the ligand-binding domains of CR2 has been published, several aspects of this structure, including the position in C3d of the binding interface, remained controversial because of disagreement with biochemical data. We now report a cocrystal structure of a CR2(SCR1-2):C3d complex at 3.2 angstrom resolution in which the interaction interfaces differ markedly from the previously published structure and are consistent with the biochemical data. It is likely that, in the previous structure, the interaction was influenced by the presence of zinc acetate additive in the crystallization buffer, leading to a nonphysiological complex. Detailed knowledge of the binding interface now at hand gives the potential to exploit the interaction in vaccine design or in therapeutics directed against autoreactive B cells.

Complement receptor 2 (CR2, CD21), found on B cells and follicular dendritic cells (FDCs), plays an important role in linking innate and adaptive immunity (1). When an antigen becomes opsonized by the complement C3 degradation product C3d (or intermediate degradation products iC3b and C3dg), the antigen moiety can bind to the B cell antigen receptor complex, while the C3d moiety simultaneously binds to the CD21/CD19/CD81 complex on the same B cell. This coligation of receptor complexes has a profound molecular adjuvant effect, lowering the threshold of antigen required for B cell activation by up to 10,000-fold (2). Additionally, the trapping of C3d-opsonized antigen by CR2 on FDCs in the lymph node is essential for generating high-affinity antibodies and memory B cells (1, 3). Whereas the CR2:C3d interaction is beneficial to the host under normal circumstances, it can also be detrimental when augmenting autoantibody responses (4, 5). This interaction must be understood at atomic level to either exploit its molecular adjuvant effect in vaccine design or to inhibit it in autoimmune circumstances. Although a cocrystal structure of a complex between C3d and the two N-terminal short consensus repeat (SCR) domains of CR2, previously established to contain the C3d binding site (6), was published in 2001 (7), the interaction surface of this structure was inconsistent with much of the biochemical data. Issues included the stoichiometry of the interaction (8), the number of SCR domains mediating the contact (9, 10), and the

contact region on C3d expected from mutagenesis data (11). Thus, the relevance of the crystallographic interaction has been controversial.

The C3d fragment of complement component C3 represents the minimal full binding-activity ligand for CR2 (6, 11, 12). The crystal structure of C3d revealed two distinct surfaces (12): (i) a convex, somewhat basic surface containing the thioester-constituting residues that mediate covalent binding to target antigen and (ii) an acidic residue-lined concave surface remote from the covalent binding site. Based on its accessible location, the known ionic strength-dependence of the CR2:C3d interaction (13, 14) and the fact that a homology model of CR2(SCR1-2) indicated prominent positively charged surface patches in both domains, the acidic pocket of C3d was considered an attractive candidate for mediating the binding interaction with CR2 (12). This hypothesis was supported by an extensive alanine mutagenic scan of surface-exposed side chains in the target area, which revealed that a number of critical residues for the interaction were predominantly located in two clusters on opposite sides of the acidic pocket (11). However, the 2001 CR2(SCR1-2):C3d cocrystal structure showed no contact with residues in the acidic pocket (7). The only interface was between the SCR2 domain and a side face of the C3d α - α 6 barrel, and this interface was unusual in that almost all interactions on the C3d side were to main-chain carbonyls, the only exception being the side chain of C3d N170 interacting with that of SCR2 Y80 (15). Not only were there no classical salt bridges, as might have been expected from the ionic strength dependence, but it was also not obvious what mediated the specificity of the interaction. Recently, we have confirmed our original findings regarding the importance of residues in the acidic pocket and ruled out any contribution of the N170 side

chain to the binding interaction (16). Furthermore, we have found that 0.2 M zinc acetate, which was used as a crystallization additive in the cocrystal structure paper, abrogated the CR2(SCR1-2):C3d binding interaction (16). We suggest that in the presence of the zinc acetate, C3d and CR2(SCR1-2) probably cocrystallized as a nonphysiologically relevant complex simply because of favorable crystal-packing energetics. Given the immunological importance of the CR2(SCR1-2):C3d interaction, we set out to obtain diffracting crystals of the complex under conditions that avoided nonphysiologic salts.

Our crystals of the human CR2(SCR1-2):C3d complex belonged to trigonal space group $R\bar{3}_2$ and diffracted to a resolution of 3.16 Å (17). The asymmetric unit contains four molecules: two CR2(SCR1-2) and two C3d molecules forming two CR2(SCR1-2):C3d complexes (Fig. 1A). The two CR2(SCR1-2):C3d complexes in the asymmetric unit are located in opposing positions where the V-shaped CR2 molecules interact head-to-head, predominantly via the SCR1 domains, similar to what has been observed in the earlier CR2(SCR1-2):C3d structure (7) [Protein Data Bank (PDB) accession code 1GHQ]. The two complexes observed in the asymmetric unit show substantial differences in temperature factors (fig. S3), and we chose the CR2(SCR1-2):C3d complex with the best electron density and lowest B-factor values for detailed analysis [i.e., complex 1; C3d (chain A) and CR2(SCR1-2) (chain C); shown in Fig. 1B]. Nevertheless, despite major differences in symmetry-related crystal contacts for the two complexes, the placement of the CR2 domains on the concave surface of C3d was the same in both complexes (fig. S4). Structural alignment of the C3d and CR2(SCR1-2) molecules in the complex with their uncomplexed counterparts (12, 18) (with respective PDB accession codes 1C3D and 1LY2) revealed no major conformational changes upon complex formation [C α root mean square deviation values of 0.5 Å for C3d and 0.9 Å for CR2(SCR1-2)]. The V-shape seen in CR2 is stabilized by tight packing interactions between SCR1 and SCR2, identical to what has been observed previously for both free CR2 (18) and the earlier structure of a complex between CR2(SCR1-2) and C3d (fig. S5) (7). It had been speculated that a normal-length carbohydrate chain attached to N107 of SCR2 would sterically prevent the adoption of such a V-shaped entity (18). However, we found that there was ample space in the complex to dock in a natural branched oligosaccharide chain (fig. S6).

In contrast to the previous structure (7), in both complexes of our asymmetric unit the V-shaped SCR domains of CR2 interact with the acidic pocket on the concave surface of C3d (Fig. 1, B and C), showing no interactions with the formerly identified interface on C3d (fig. S7). The buried surface area (BSA) of the complex (i.e., total of both sides of

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the interface) is $2250 \text{ \AA}^2$, and the prominent shape complementarity observed in the complex is illustrated in Fig. 2A. Using the same BSA calculations, the interface in the previously reported CR2(SCR1-2):C3d complex (7) was less than $800 \text{ \AA}^2$. This is comparable to the nonphysiologic crystallization-induced interactions observed between the two SCR1 domains ($880 \text{ \AA}^2$) in both structures, given the solution data, which clearly indicate that CR2(SCR1-2) does not readily dimerize (8).

Contacts between C3d and SCR1 account for 75% of the total BSA of the complex (Fig. 2A). These contacts include specific interactions involving salt bridges and hydrogen bonds between the side chains of residues R13 (SCR1) and E37 (C3d) and between SCR1 residues R28 and K41 with D163 in C3d (Figs. 1C and 2B and table S2A). Further side chain–side chain interactions involve hydrogen bonds between Y16 (SCR1) and Q50 (C3d) and between S18 (SCR1) with K57 (C3d). The shape and charge

complementarity between SCR1 and C3d is supplemented by van der Waals interactions between V26 (SCR1) and a clearly visible pocket in the concave surface of C3d lined with residues V97, I100, A101, I102, D103, S104, and I164 (Figs. 1C and 2, A and B). In contrast, there are only a few interactions between C3d and SCR2 (Fig. 2, A and B, and table S2, A and B), including R83 (SCR2), interacting with C3d residues K162, Q205, and Q249, as well as D92 (SCR2) and K108 (SCR2), which are involved in charge interactions with K251 and E166 in C3d, respectively.

As annotated in tables S2A and S2B, existing biochemical data support a large number of the interactions that we observe between C3d and SCR1 (11, 16, 19, 20). In contrast, there was little supporting data for the SCR2–C3d interactions we observed. Thus, we used alanine mutagenesis to test the importance of the side chains of R83, D92, and K108 to the binding interaction. A sample set of sensorgrams

of $0.25 \text{ }\mu\text{M}$ CR2(SCR1-2) variants injected as analytes over a C3d-coupled biosensor chip are shown in Fig. 3A, and full concentration-dependent binding curves are shown in Fig. 3B. Relative to wild-type (WT) CR2(SCR1-2), there is a fourfold decrease in the binding affinity of the $\text{Lys}^{108} \rightarrow \text{Ala}^{108}$ (K108A) mutant (Fig. 3B), consistent with the importance of the observed salt bridge. An alanine mutation of the C3d partner residue, E166, was previously found to cause a $\sim$ sixfold decrease in binding to CR2 (11, 16). The $\sim$ twofold decrease in binding affinity for the R83A mutant is consistent with the interactions that we observed (table S2, A and B) and is quantitatively within the range of defect observed previously for alanine and charge reversal mutants of this residue (19). In contrast, only a modest $\sim$ 20% defect in binding affinity is observed for the D92A mutant, suggesting that the salt bridge observed in the structure with C3d side chain K251 is not essential for the binding inter-

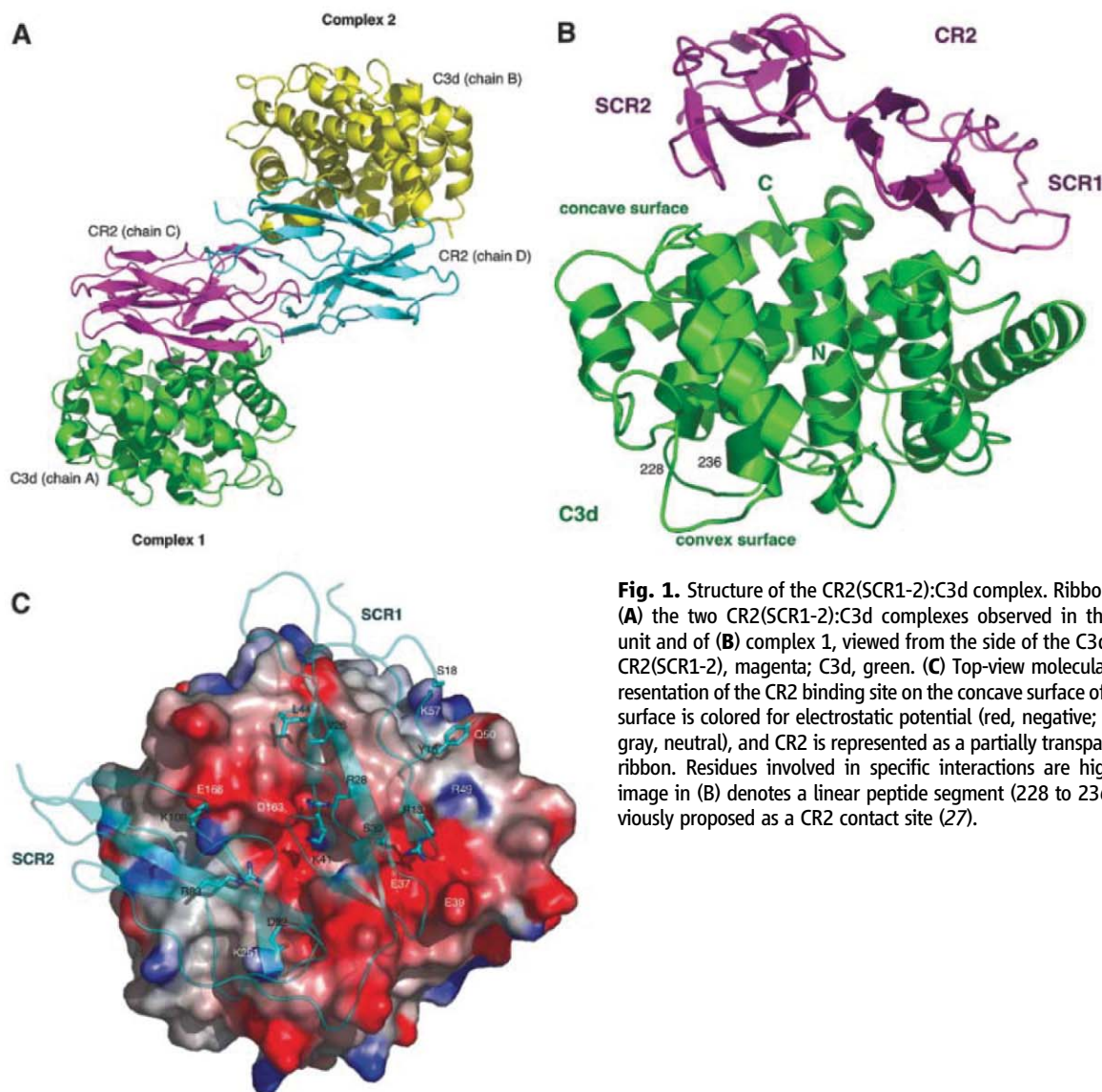


Fig. 1. Structure of the CR2(SCR1-2):C3d complex. Ribbon diagrams of (A) the two CR2(SCR1-2):C3d complexes observed in the asymmetric unit and of (B) complex 1, viewed from the side of the C3d α - α 6 barrel. CR2(SCR1-2), magenta; C3d, green. (C) Top-view molecular surface representation of the CR2 binding site on the concave surface of C3d. The C3d surface is colored for electrostatic potential (red, negative; blue, positive; gray, neutral), and CR2 is represented as a partially transparent turquoise ribbon. Residues involved in specific interactions are highlighted. The image in (B) denotes a linear peptide segment (228 to 236) in C3d previously proposed as a CR2 contact site (27).

action. The fact that C3d K251 is seen to make interactions with at least five other SCR2 residues in addition to D92 (table S2B), whereas SCR2 K108 only has C3d E166 as an interaction partner, probably explains the difference we observe for the relative importance to the binding strength of these two SCR2-C3d salt bridges. Finally, it is worth noting that although both the structural and mutagenesis data presented here were obtained using *Escherichia coli*-derived (and, therefore, nonglycosylated) CR2(SCR1-2), the corroborating literature data referred to regarding the effects of mutations at SCR2 R83

(19) and C3d E166 (11, 16) were obtained using CR2 with mammalian glycan chains. This further supports the physiologic relevance of the SCR2:C3d interactions observed in our structure. Our structure of the CR2(SCR1-2) complex is consistent with a number of other biochemical studies. First, the previously reported ability of the *Staphylococcus aureus*-derived protein fragments Sbi-IV (16, 21) and Efb-C (22) to inhibit the CR2(SCR1-2):C3d interaction is readily understood in terms of the substantially overlapping contact areas of these ligands on C3d (23, 24)

(fig. S8). Second, whereas iC3b, C3dg, and C3d have indistinguishable binding affinities for CR2 (6, 11, 12), the ~10-fold weaker binding interaction with C3b (6, 14) is consistent with a steric clash that would occur between the β chain MG1 domain of the C3b structure (25) and an N-terminal loop of SCR1 (L10 to Y16), assuming that all SCR1 interactions seen in binding to free C3d also take place on C3b (fig. S9). Although the MG1 domain is still present in iC3b, electron microscopy evidence suggests that the C3d domain of iC3b has moved away from the β chain ring, thus relieving the steric clash (26).

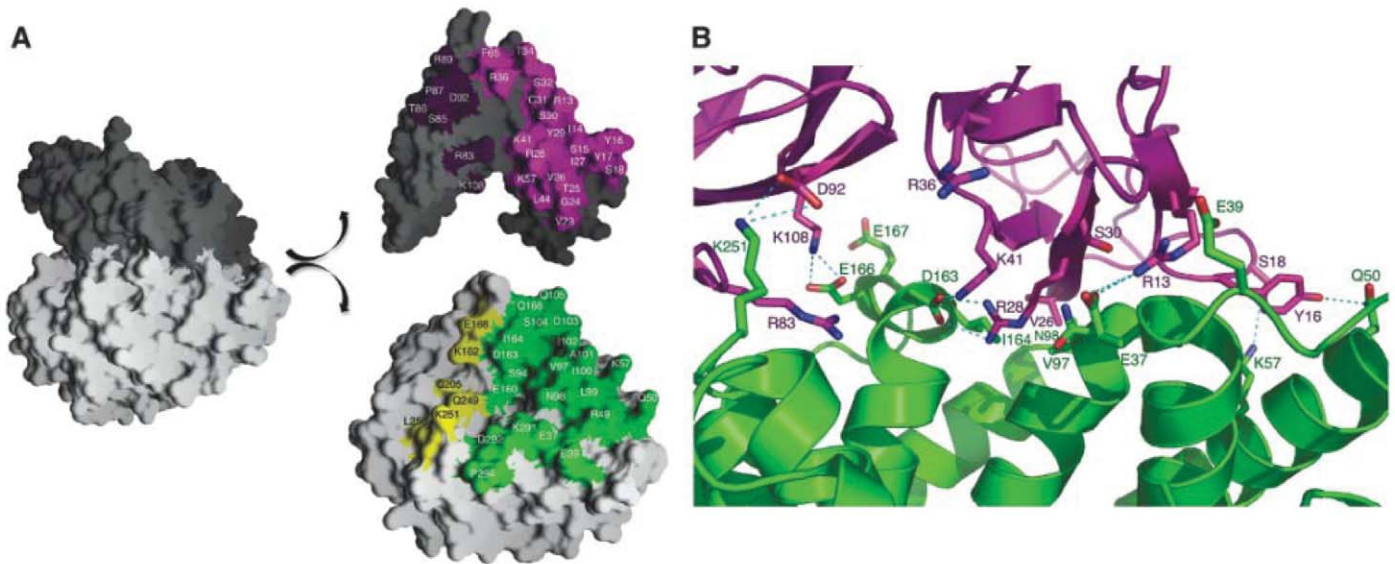


Fig. 2. Interaction surface of the CR2(SCR1-2):C3d complex. **(A)** Surface representations of CR2 (dark gray) and C3d (light gray) illustrating their contributions to the interaction surface. In a splayed-apart view, domain-specific contributions to the interaction surface are shown on both molecules, with SCR1 (magenta) and SCR2 (dark plum) on the CR2(SCR1-2) surface and SCR1 (green) and SCR2 (yellow) on the C3d surface. **(B)** Close-up view of the interactions between CR2(SCR1-2) (magenta) and the concave surface of C3d (green). The specific side chain-side chain interactions depicted here are discussed in the text. The α 12 helix of C3d has been removed for presentation clarity. Dashed lines indicate atoms interacting through salt bridges or hydrogen bonds.

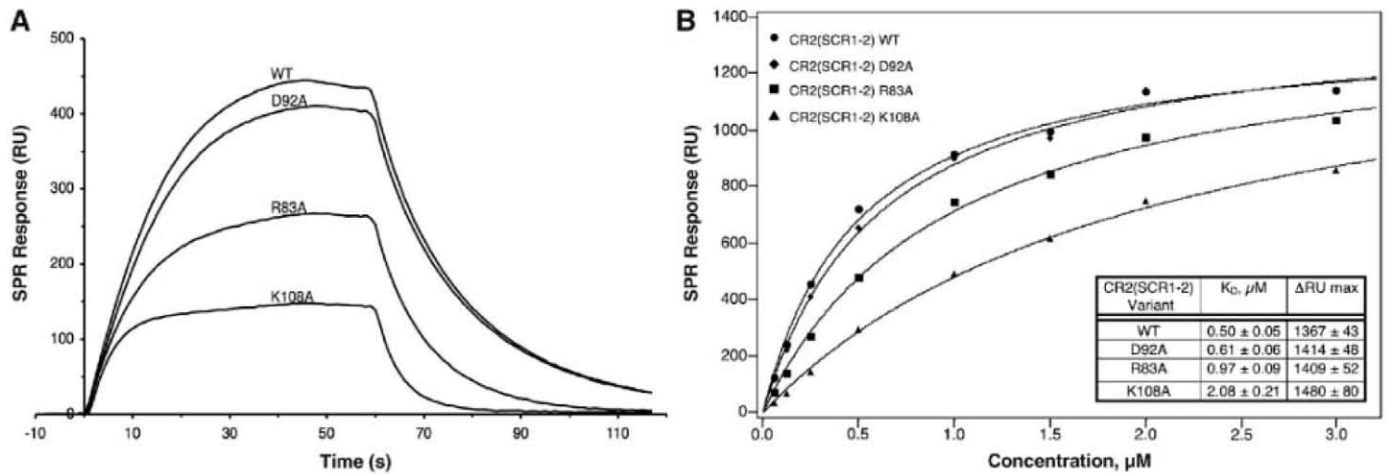


Fig. 3. Assessment by surface plasmon resonance (SPR) of the binding of WT and mutant CR2(SCR1-2) to biosensor-coupled C3d. **(A)** Overlay of sensorgrams representing the WT and three mutant CR2(SCR1-2) variants at 0.25 μ M analyte concentration. The change in resonance units (RU) shown is after subtraction of the reference channel. **(B)** Full binding curves

derived from the steady-state plateau change in RU of each sensorgram as a function of CR2(SCR1-2) concentration. Nonlinear regression fits to a 1:1 Langmuir binding isotherm are shown. (Inset) Derived binding parameters. Error estimates of parameters are those of the fit procedure. K_D , dissociation constant.

Third, a synthetic peptide comprising C3d residues 216 to 243 was previously reported to bind to CR2 (27, 28), with residues 228 to 239 suggested as the CR2 contact site in C3d (27). However, using extensive mutagenesis, we failed to demonstrate a major role for this segment in mediating the interaction of CR2 with either iC3b or C3dg (14). Consistent with this, the contact region on C3d for CR2(SCR1-2) observed in our new cocrystal structure is far removed from the 228-to-239 peptide segment (Fig. 1B). Finally, a C3d K162A mutation was previously noted to produce a ~twofold gain in CR2 binding activity (11). This may be rationalized in terms of relief of mutual charge repulsion that would occur in the WT C3d case because of the proximity (4.5 Å) of the positively charged ϵ -amino group of C3d K162 to the positively charged guanido group of SCR2 R83 (fig. S10).

A recent study (29) has made use of mutagenesis data-constrained molecular-docking programs to calculate six “high-scoring” models of the CR2(SCR1-2):C3d complex. All models placed both the SCR1 and SCR2 domains on the concave surface of C3d. However, no atomic-level detail regarding specific interactions could be deduced from the models. The atomic-level detail in the cocrystal structure presented here should enable the rational design of compounds that would either inhibit the CR2:C3d interaction in an autoreactive B cell disease situation or be used to decorate antigens in an attempt to mimic the molecular adjuvant properties of antigen-associated C3d oligomers (2) in a vaccine situation.

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Supporting Online Material

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Materials and Methods

Figs. S1 to S10

Tables S1 and S2

References

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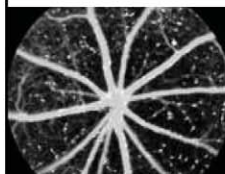
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Further information about LV is available at www.niaid.nih.gov/labs/aboutlabs/lv, information about DIR laboratories is available at www.niaid.nih.gov/about/organization/dir, and information about working at NIAID is available at www.niaid.nih.gov/careers/ve.



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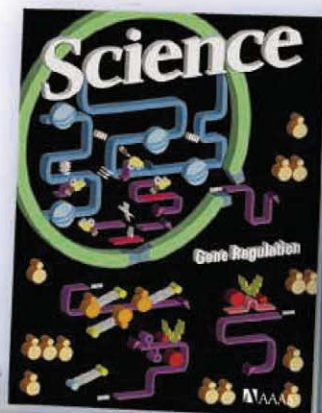
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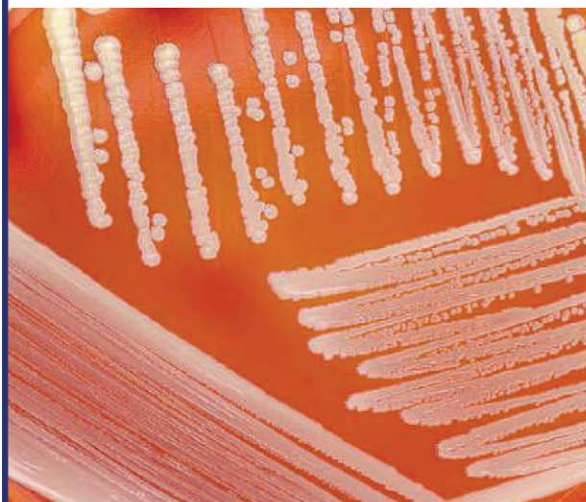


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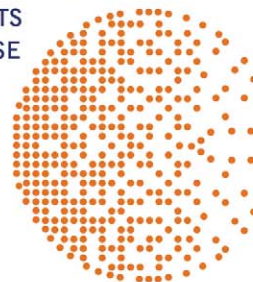
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- Do academic scientists have less work/life balance?

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PRIZES



KUWAIT PRIZE 2011 Invitation for Nominations

The **Kuwait Foundation for the Advancement of Sciences (KFAS)** institutionalized the **KUWAIT Prize** to recognize distinguished accomplishments in the arts, humanities and sciences. The Prizes are awarded annually in the following categories:

- A. Basic Sciences
- B. Applied Sciences
- C. Economics and Social Sciences
- D. Arts and Literature
- E. Arabic and Islamic Scientific Heritage

The Prizes for **2011** will be awarded in the following fields:

- | | | |
|--|---|-------------------------------------|
| 1. Basic Sciences | : | Physiology |
| 2. Applied Sciences | : | Nuclear Technology |
| 3. Economic and Social Sciences | : | Financial Markets in the Arab World |
| 4. Arts and Literature | : | Novels |
| 5. Arabic and Islamic Scientific Heritage | : | Astronomy |

Foreground and Conditions of the Prize:

1. Two prizes are awarded in each category:
* A Prize to recognize the distinguished scientific research of a Kuwaiti citizen, and,
* A Prize to recognize the distinguished scientific research of an Arab citizen.
2. The candidate should not have been awarded a Prize for the submitted work by any other institution.
3. Nominations for these Prizes are accepted from individuals, academic and scientific centers, learned societies, past recipients of the Prize, and peers of the nominees. No nominations are accepted from political entities.
4. The scientific research submitted must have been published during the last ten years.
5. Each Prize consists of a cash sum of K.D. 30,000/- (approx. U.S.\$100,000/-), a Gold medal, a KFAS Shield and a Certificate of Recognition.
6. Nominators must clearly indicate the distinguished work that qualifies their candidate for consideration.
7. The results of KFAS decision regarding selection of winners are final.
8. The documents submitted for nominations will not be returned regardless of the outcome of the decision.
9. Each winner is expected to deliver a lecture concerning the contribution for which he was awarded the Prize.

Inquiries concerning the KUWAIT PRIZE and nominations including complete curriculum vitae and updated lists of publications by the candidate with **four copies** of each of the published papers should be received before **01/12/2011** and addressed to:

The Director General

The Kuwait Foundation for the Advancement of Sciences - P.O. Box: 25263, Safat - 13113, Kuwait.

Tel: (+965) 22429780 / Fax: 22403891 / E-Mail: prize@kfass.org.kw

POSITIONS OPEN



南京医科大学生殖医学国家重点实验室 (筹)

STATE KEY LABORATORY OF REPRODUCTIVE MEDICINE, NANJING MEDICAL UNIVERSITY, CHINA

Full /Associate Professor Positions State Key Laboratory of Reproductive Medicine, Nanjing Medical University

The State Key Laboratory of Reproductive Medicine (SKLRM) at Nanjing Medical University was approved for construction in 2011. Now we sincerely invite applications for full-time faculty positions, at academic ranks of Associate or Full Professor. Individuals with demonstrated accomplishments in, but not limited to, the following areas are encouraged to apply: reproductive biology, reproductive endocrinology, reproductive pathology, reproductive toxicology and reproductive epidemiology.

SKLRM's major research orientations cover the following: (1) mechanisms of gametogenesis and maturation, (2) environment - gene interaction effects on gametogenesis, (3) infertility and maternal and child health, and (4) molecular mechanisms of reproductive-related diseases. At present SKLRM has established a series of excellent platforms including proteomic platform, gametes and embryos operation platform, platform for detection of environmental endocrine disruptors, etc. In addition, our laboratory was also equipped with AAALAC accredited SPF animal facility, which could support the functional analysis of transgenic and knockout animals. Highly competitive research support will be provided in a vigorous and stimulating environment. Individuals will have an opportunity to establish a state-of-the-art independent research program in newly renovated space and to get startup research funds. More information can be found at <http://reprod.njmu.edu.cn/keylab/>.

Interested individuals, regardless of their nationalities, should submit a detailed letter of interest, curriculum vitae, PDFs of three of their best publications, and two letters of recommendation to: **Jiahao Sha Ph.D.** (shajh@njmu.edu.cn) or **Ran Huo Ph.D.** (huoran@njmu.edu.cn), State Key Laboratory of Reproductive Medicine, Nanjing Medical University, 140 Hanzhong Road, Nanjing, Jiangsu 210029, China. The positions are available immediately. Applications will be evaluated by faculty search committee upon receipt until the positions are filled.

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AAAS is here – connecting government to the scientific community.

As a part of its efforts to introduce fully open government, the White House is reaching out to the scientific community for a conversation around America's national scientific and technological priorities.

To enable the White House's dialogue with scientists, AAAS launched Expert Labs, under the direction of blogger and tech guru Anil Dash. Expert Labs is building online tools that allow government agencies to ask questions of the scientific community and then sort and rank the answers they receive.

On April 12, 2010, AAAS asked scientists everywhere to submit their ideas to the Obama administration and at the same time launched the first of Expert Labs tools, Think Tank, to help policy makers collect the subsequent responses. The result was thousands of responses to the White House's request, many of which are already under consideration by the Office of Science and Technology Policy.

As a AAAS member, your dues support our efforts to help government base policy on direct feedback from the scientific community. If you are not already a member, join us. Together we can make a difference.

To learn more, visit aaas.org/plusyou/expertlabs





FACULTY POSITION in Cardiovascular Regenerative Medicine

The Cardiovascular Innovation Institute (CII) invites applications for a tenured/tenure-track faculty position in the field of Cardiovascular Regenerative Medicine. Applicants are expected to have academic and/or industrial experience indicating promise of an exceptional future in interdisciplinary translational cardiovascular research and discovery. Investigators with experience in Regenerative Medicine and with experience and interest translating research to clinical applications are encouraged to apply.

The Cardiovascular Innovation Institute is a non-profit partnership between the University of Louisville and Jewish Hospital dedicated to researching, developing, and testing the next generation of heart and vascular health care solutions. Institute investigators share appointments with the UofL and work in a new state-of-the-art research building in a highly collaborative environment with excellent opportunities to translate research through clinical partnerships and industrial collaborations.

The CII is located in Louisville, Kentucky a progressive city situated along the scenic Ohio River with a thriving cultural scene and a variety of outdoor recreation opportunities.

Applicants should submit a cover letter describing research interests and curriculum vitae to: **Stuart K. Williams, Ph.D., Executive and Scientific Director, Cardiovascular Innovation Institute, Louisville, KY, 40202** or electronically to **e-mail: stu.williams@louisville.edu**. Consideration of applications will begin immediately and the position will be open until filled.

The University of Louisville is an Affirmative Action, Equal Opportunity, Americans with Disabilities Employer, committed to diversity, and in that spirit, seeks applications from a broad variety of candidates.

POSTDOCTORAL POSITION available to work on (1) roles of Ubiquitin Pathway in Regulation of Cell Cycle, or Differentiation or (2) Changes in Ubiquitin conjugate dynamics in response to stress. Tufts University Medical School is a highly collaborative and productive environment, located in downtown Boston. We are about 20 minutes from Harvard, MIT, and Boston University. Candidates should have at least two English language first-authored papers. Send curriculum vitae, a list of grades received in graduate science courses and a two-page research proposal of how your research would further the objectives of our group, as indicated above, to: **Allen Taylor, Laboratory for Nutrition and Vision Research, HNRCA, Tufts University, 711 Washington Street, Boston, MA 02111, telephone: 617-556-3156, fax: 617-556-3132, website: <http://www.tufts.edu/~ataylo01/>.**

POSTDOCTORAL POSITION

A postdoctoral position is available to work on molecular aspects of cell cycle deregulation by viral oncoproteins. A strong molecular biology expertise is required. Send resume and names of three references electronically to **Dr. Bayar Thimmapaya, professor, Microbiology and Immunology Department, Northwestern University Medical School, Chicago. E-mail: b-thimmapaya@northwestern.edu.**

Northwestern University is an Affirmative Action/Equal Opportunity Employer. Women and minorities are encouraged to apply. Hiring is contingent upon eligibility to work in the United States.



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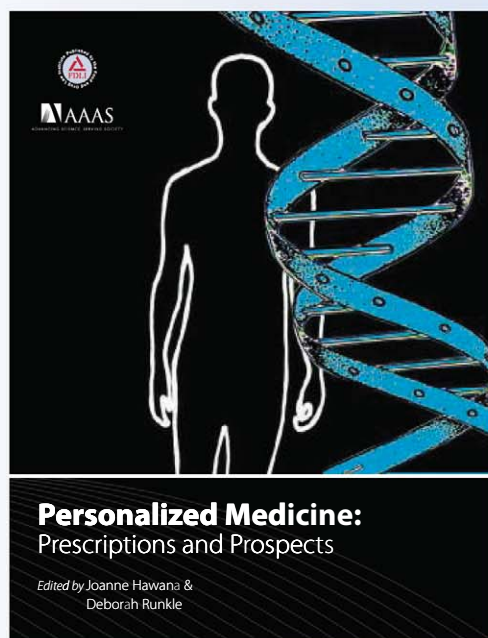


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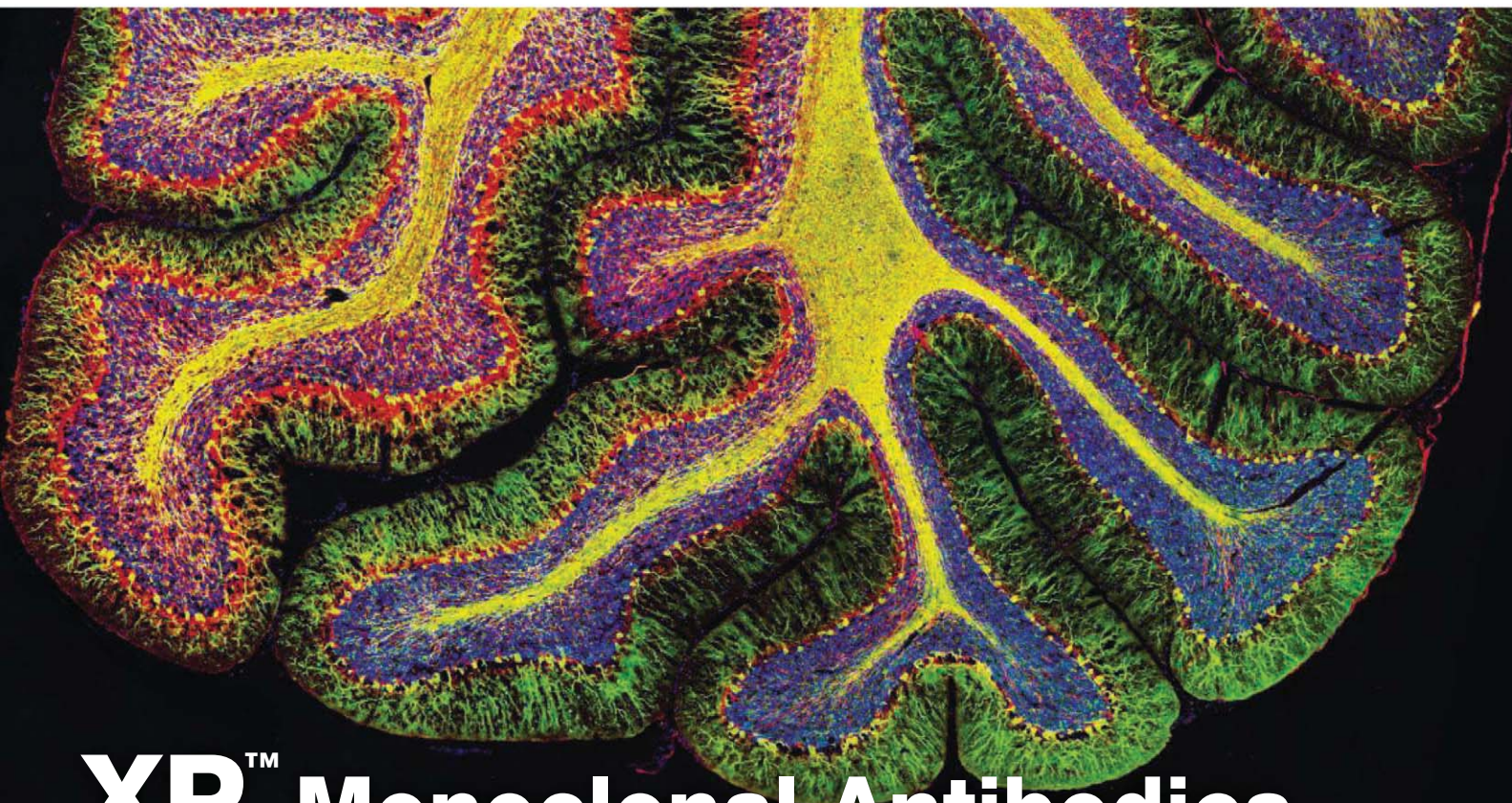
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